PERKIN ELMER

Lambda 12
Lambda 14
Lambda 14P

UV/Vis Spectrometers

Operation

Part Number 0993-5011
Publication B2161.20
Release 2.0/Feb. 95

UV/Vis/NIR Spectroscopy

Certificate No. FM 22178

Bodenseewerk Perkin-Elmer GmbH is registered for the design and manufacture of laboratory analytical equipment in respect to the quality system according to ISO 9001: 1987/EN 29001: 1987/BS 5759: Part 1: 1987.

Perkin-Elmer and UV WinLab are trademarks of the Perkin-Elmer Corporation

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

Release History

Release	Publication Date
5X	June 1994
1.0	September 1994
2.0	February 1995

The information contained in this document is subject to change without notice.

This document contains proprietary information that is protected by copyright. All rights are reserved. No part of this document may be reproduced in any form whatsoever or translated into any language without the prior written permission of the Perkin-Elmer Corporation or one of its subsidiaries.

Copyright © 1994 Bodenseewerk Perkin-Elmer GmbH

Printed in the Federal Republic of Germany

Technical Documentation
Bodenseewerk Perkin-Elmer GmbH
D-88647 Ueberlingen, Federal Republic of Germany

Part Number: 0993-5011
Publication: B2161.20
Release: 2.0/ February 1995

Table of Contents

Safety Information

Safety Conventions in the Handbook S-2
IEC 1010 – Safety Requirements S-3
Electricity S-4
Radio Frequency S-5
Environment S-6
Chemicals S-6
Waste Disposal S-6
UV Radiation S-7
Toxic Fumes S-7
Compressed Gases S-7

Chapter 1: Introduction

1.1 Keys 1-2
1.1.1 Key Combinations 1-3
1.1.2 Displays 1-4

Chapter 2: Switching On and Off

2.1 Startup 2-2
2.2 Shutdown 2-3
2.3 Single Cell Holder 2-4
2.3.1 Description 2-4
2.3.2 Installing the Single Cell Holder 2-5
2.3.3 Aligning the Single Cell Holder 2-6
2.3.4 Minimum Volume Applications 2-9

Chapter 3: Operating without Methods

3.1 Overview ... 3-1
3.2 Setting the Wavelength Manually ... 3-2
3.3 Manual Background Correction ... 3-3
3.4 Quick Sample Measurement ... 3-4
3.5 Reset ... 3-5

Chapter 4: How to use Methods

4.1 What are Methods? ... 4-1
4.2 Selecting a Method ... 4-2
4.2.1 Default Methods ... 4-3
4.3 Editing Methods ... 4-4
4.3.1 Modifying a Method ... 4-5
Changing a Parameter ... 4-6
Tagging a Parameter ... 4-7
4.3.2 Deleting a Method ... 4-8
4.3.3 Creating a New Method ... 4-9
Creating an Empty Method File ... 4-9
Overwriting an Existing Method ... 4-10
4.3.4 New Method Name ... 4-11
4.3.5 Checking a Method ... 4-12
4.3.6 Copying Method Parameters into a New Method File ... 4-13
Marking for Copy ... 4-13
Copying the Method into Another Method File ... 4-14
4.3.7 Printing Out a Method ... 4-15
4.4 Printing Out a Directory ... 4-16
4.4.1 Spectrometer Directory ... 4-16
4.4.2 Branch Directory ... 4-16
4.5 Help Key ... 4-17

Chapter 5: Measuring using methods

5.1 Overview ... 5-1
5.2 Method Procedure ... 5-2
5.3 Analysis Procedure ... 5-3

5.4 Single Wavelength Measurements 5-6
5.5 Scanning a Spectrum 5-8
5.6 Measurement at Several Wavelengths 5-10
5.7 Concentration Determination 5-12
5.7.1 CONCENTRATION 1 Method (Peak heights) 5-12
5.7.2 CONCENTRATION 2 Method (Peak Areas, 2nd Derivative) ... 5-18
5.7.3 Processing the Calibration Curve (CONCENTRATION Methods) 5-24
5.8 Enzyme Kinetics 5-26
5.9 Substrate Kinetics 5-30
5.9.1 Recalculation of Results with POSTRUN KIN 5-34
5.10 Methods for Quantitative Analysis of Oligonucleotides 5-36
5.11 Date/Time 5-40
5.12 Wakeup 5-42
5.13 Self Test 5-43

Chapter 6: Operating with accessories

6.1 General 6-1
6.2 Accessories 6-1
6.3 Requirements for Operation with Accessories 6-2
6.4 Using Methods with Accessories 6-2
6.4.1 Spectrometer without Accessory Board 6-3
6.5 Running Methods with an Accessory 6-4
6.6 Background Correction with Cell Changers 6-5
6.7 CONCENTRATION Methods with Cell Changers 6-6
6.8 Accessory Parameters 6-7

Chapter 7: Care

7.1 Daily Care 7-1
7.2 Use and Care of Cells 7-2
7.2.1 Cell Handling 7-2
7.2.2 Pressure Buildup in Cells 7-3
7.2.3 Sample Compartment Windows 7-3

Chapter 8: Analytical Notes

8.1 Background Correction 8-1
8.2 Unusual Samples 8-2
8.3 Solvent Properties 8-4

Chapter 9: Error Messages

9.1 Error Messages Shown on the Display 9-1
9.2 Error Reports on the Printer 9-6

Chapter 10:Parameter Numbers and Descriptions

10.1 Parameter Numbers and Descriptions 10-1

Appendix 1:SuperUser

A1.1 Activating SuperUser Mode A1-1
A1.2 Deactivating SuperUser Mode A1-1

Appendix 2:Protect Functions

A2.1 General Information about Protect Functions A2-1
A2.2 Setting Protect Functions A2-2
Setting Method Protect Function A2-3

Appendix 3:Instrument Branches

A3.1 Overview Of the Instrument Branches A3-1
A3.2 Selecting a Branch A3-2
A3.3 About the Various Branches A3-3
A3.4 APPLICATION - the Application Branch A3-3
A3.5 COMMUNICATION - the Communication Branch A3-3
A3.6 CALIBRATION - the Calibration Branch A3-4
A3.7 CONFIGURATION - the Configuration Branch A3-5
A3.8 VALIDATION - the Validation Branch A3-7
A3.9 TEST - the Test Branch A3-7

Appendix 4: Enzyme Kinetics

A4.1 Enzymatic Analytical Procedures A4-1
A4.2 Enzyme Kinetics A4-2
A4.3 Substrate Kinetics A4-4

Annex 1: Translations of Warnings

Illustrations

1-1 Features common to Lambda 12, 14 and 14P 1-1
2-1 Single Cell Holder B050-5071 2-4
2-2 Correct Alignment of the Radiation Beam in the Cell Sample Area 2-7
5-1 Flow diagram of a typical method procedure 5-4

Tables

2-1 Minimum Volume Requirements 2-9
4-1 Text and symbols on the lower line of the display. 4-6
4-2 Type of Tags 4-7
4-3 Help Key Combinations 4-17
6-1 BACK CORR Tagging 6-5
8-1 Lower Wavelength Limits of Solvents 8-5
A2-1 Protect Functions A2-1

Safety Information

This section contains important safety information. Please read this information carefully before you install the instrument and observe the recommendations at all times.

Safety Information

Contents

Section	Page
Safety Conventions in the Handbook	S-2
IEC 1010 – Safety Requirements	S-3
Electricity	S-4
Radio Frequency	S-5
Environment	S-6
Chemicals	S-6
Waste Disposal	S-6
UV Radiation	S-7
Toxic Fumes	S-7
Compressed Gases	S-7

Safety Conventions in the Handbook

Possible hazards that could harm the user or result in damage to the instrument are clearly stated at appropriate places throughout this handbook.

The following safety conventions are used throughout this handbook:

W01.01

Warning
We use the term WARNING to inform you about situations that could result in ***personal injury*** *to yourself or other persons.*
Details about these circumstances are in a box like this one.

C01.01

Caution
We use the term CAUTION to inform you about situations that could result in ***serious damage to the instrument*** *or other equipment.*
Details about these circumstances are in a box like this one.

Translations of the warning messages used in this handbook are given in an Annex at the end of this handbook.

IEC 1010 – Safety Requirements

This instrument has been designed and tested in accordance with Perkin-Elmer specifications and IEC 1010: *Safety requirements for electrical equipment for measurement, control, and laboratory use.* The instrument is protected in accordance with IEC Class 1 rating.

This handbook contains information and warnings that must be followed by the user to ensure safe operation and to maintain the instrument in a safe condition.

Before using this instrument it is essential to read the handbook carefully and to pay particular attention to any advice it contains concerning potential hazards that may arise from the use of the instrument. The advice is intended to supplement, not supersede, the normal safety code of behavior prevailing in the user's country.

Electricity

To ensure satisfactory and safe operation of the instrument, it is essential that the green/yellow lead of the line power cord is connected to true electrical earth (ground).
If any part of the instrument is not installed by a Perkin-Elmer service representative, make sure that the line power plug is wired correctly:

Terminal	Cord Lead Colors	
	International	USA
Live	Brown	Black
Neutral	Blue	White
Protective Conductor (earth/ground)	Green/Yellow	Green

W02.01

WARNING

Electrical Hazard

Any interruption of the protective conductor inside or outside the instrument or disconnection of the protective conductor (earth/ground) terminal is likely to make the instrument dangerous.
Intentional interruption is prohibited.

W02.02

Lethal voltages are present in the instrument

- *Even with the power switch OFF, line power voltages can still be present within the instrument.*
- *When the instrument is connected to line power, terminals may be live, and opening covers or removing parts (except those to which access can be gained by hand) is likely to expose live parts.*
- *Capacitors inside the instrument may still be charged even if the instrument has been disconnected from all voltage sources.*

When working with the instrument:

- Connect the instrument to a correctly installed line power outlet that has a protective conductor (earth/ground).
- Do not attempt to make internal adjustments or replacements except as directed in this handbook.
- Do not operate the instrument with any covers or parts removed.
- Servicing should be carried out only by a Perkin-Elmer Service Representative or similarly authorized and trained person.
- Disconnect the instrument from all voltage sources before opening it for any adjustment, replacement, maintenance, or repair. If, afterwards, the opened instrument must be operated for further adjustment, maintenance, or repair, this must only be done by a skilled person who is aware of the hazard involved.
- Use only fuses with the required current rating and of the specified type for replacement. **Do not use makeshift fuses or short circuit the fuse holders.**
- Whenever it is likely that the instrument is no longer electrically safe for use, make the instrument inoperative and secure it against any unauthorized or unintentional operation.

  The instrument is likely to be electrically unsafe when it:
  - Shows visible damage.
  - Fails to perform the intended measurement.
  - Has been subjected to prolonged storage under unfavorable conditions.
  - Has been subjected to severe transport stresses.

Radio Frequency

This equipment generates radio frequency energy and if not installed and used properly, i.e. in strict accordance with the handbook, may cause harmful interference to radio communications.

Environment

W01.03

Explosive Atmosphere
This instrument is not designed for operation in an explosive atmosphere.

The instrument will operate correctly under the following conditions:

- Indoors.
- Temperature +15 °C to +35 °C.
- Relative humidity 20 % to 80 %.

Chemicals

Use, store, and dispose of chemicals that you require for your analyses in accordance with the manufacturer's recommendations and local safety regulations.

Waste Disposal

Waste containers may contain corrosive or organic solutions and small amounts of the substances that were analyzed.
If these materials are toxic, you may have to treat the collected effluent as hazardous waste. Refer to your local safety regulations for proper disposal procedures.

Deuterium lamps are maintained under reduced pressure. When you dispose of lamps that are defective or otherwise unusable, handle them correctly to minimize the implosion risk.

UV Radiation

You should be aware of the health hazard presented by UV radiation.

- When the deuterium (UV) lamp is switched on, do not open spectrometer covers unless specifically instructed to do so in the handbook.
- Always wear UV-absorbing eye protection when the deuterium lamp is exposed.
- *Never* gaze into the deuterium lamp.

Toxic Fumes

If you are working with volatile solvents, toxic substances, etc., you should provide an efficient laboratory ventilation system to remove vapors that may be produced when you are performing analyses.

Compressed Gases

Handle cylinders of compressed gas with care, in accordance with local regulations.
We recommend that gas cylinders be located outside the laboratory and the gases led to the laboratory through approved gas supply lines.
Use only approved tubing, connectors, and regulators for gas supply lines.

Introduction 1

Introduction 1

The Lambda 12, Lambda 14 and Lambda 14P are versatile spectrometers operating in the ultraviolet (UV) and visible (Vis) spectral ranges. The spectrometers have some common features.

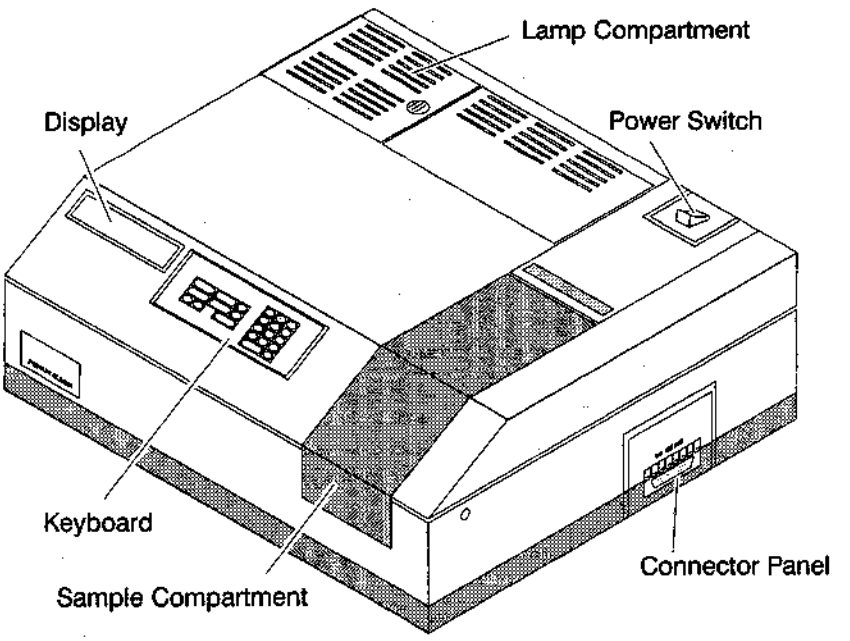
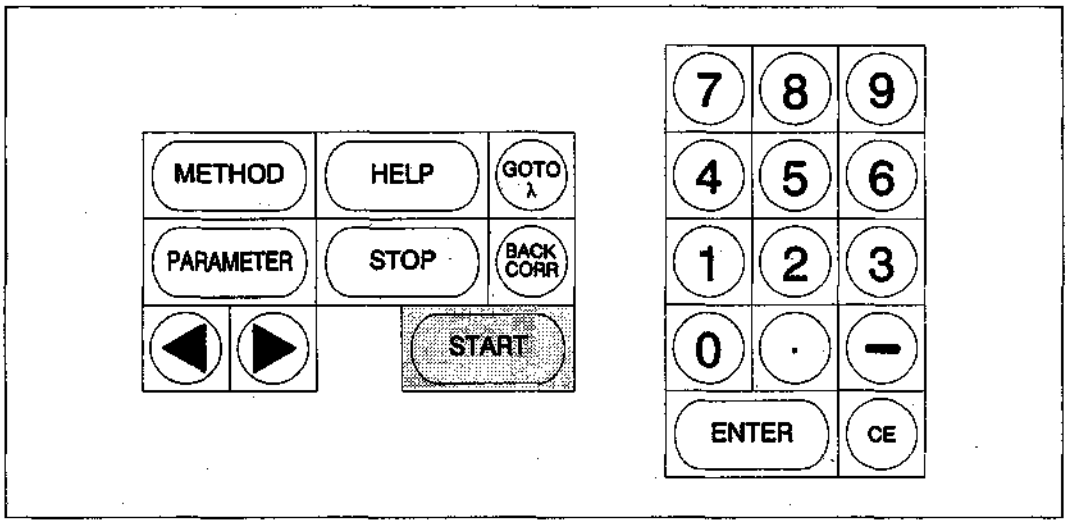


Figure 1-1 Features common to Lambda 12, 14 and 14P

1.1 Keys



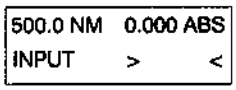
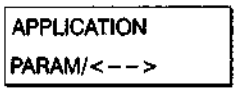
Key	Description
[METHOD]	Selects methods. Use with numerical keys (*see page 4-2*).
[HELP]	Provides additional parameter information on the display.
[GOTO λ]	To change the wavelength setting (*see page 3-2*).
[PARAMETER]	Selects next parameter. Switches to next lower level.
[STOP]	Stops a method. Switches to next higher level.
[BACK CORR]	Starts background correction (*see page 3-3*).
[◀] [▶]	Selects previous or next element in a particular level.
[START]	To start and continue a method.
[0] to **[9]**	Numerical keys.
[•]	Decimal point.
[−]	Minus, used to enter negative values.
[ENTER]	Confirms parameter.
[CE]	Clears unconfirmed parameter entry.

1.1.1 Key Combinations

Key Combinations	Description
[nnn] [METHOD]	Selects method nnn.
[nnn.n] [GOTO λ]	To change to wavelength nnn.n.
[nn] [PARAMETER]	Selects parameter nn when you are in the parameter list level.
[•] [PARAMETER]	Selects parameter tagging. Selects default methods from Application level.
[−] [PARAMETER]	Selects previous parameter.
[•] [HELP]	Prints out a method or branch directory.
[0] [HELP]	Prints out current values shown on the display.
[1] [HELP]	Prints out method parameters.
[2] [HELP]	Prints out a directory of the methods available in the branch.
[3] [HELP]	Prints out the additional method information shown on the display.
[4] [HELP]	Line feed.
[5] [HELP]	Form feed.
[6] [HELP]	Prints out the Peltier cell holder temperature shown on the display. *Functions only when the Peltier accessory is installed.*
[7] [HELP]	Prints out the spectrometer status.
[7] [9] [•]	Full reset (*see page 3-5*). (*Caution: all methods are deleted!*).
[1] [5] [−]	Switches to Super User mode (*see page A1-1*).

1.1.2 Displays

This section contains a summary of the most common displays.

Display	Description
 Standby display	Standby display with wavelength and measured value. Starting point, appears after switch-on following initialization routine. → Set up absorbance manually, set wavelength manually, select method, print out method directory of the relevant branch, return to branch header.
	Branch header with branch name. → Select the branch method, print out the spectrometer directory, change to another branch, select default method of branch, return to standby display.
	Method header with method number and type. → Start method, select method processing, select another method, return to standby display.
	Method processing with selected processing function. → Start processing function, select another processing function, return to method header.
	Parameter directory with parameter names and value. → Change parameter, select next/previous parameter, start method, select help messages for current parameter, return to method header.

Displays shown during the measurement:

Display	Description
BACK CORR PRESS START	Request to start background correction.
BACK CORR xxx nm xxx ABS	Display during background correction.
n SAMPLE n PRESS START	Request to start sample measurement.
SCAN CYCLES xxx nm xxx ABS	Display during sample measurement (SCAN method).
ORDINATE & ABS <--->	Display of a tagged parameter. If necessary, the parameter can be changed.
BACK CORR IN CELL1 PRESS START	Cell Changer: request to insert blank solutions and start background correction.
SAMPLES IN 9-CELL PRESS START	Cell Changer: request to insert sample solutions and start the measurement.
BACK CORR SIPPER ACCESSORY START	Sipper: request to press start key on the Sipper (background correction).
SAMPLE 1 SIPPER ACCESSORY START	Sipper: request to press start key on the Sipper (sample measurement).
REF 1 [xxx] PRESS START	Request to measure a reference solution.

Switching On and Off 2

Startup and Shutdown 2

Please take your time when operating the spectrometer, wait until the BUSY display has switched off before pressing the next key in the sequence. This allows the software to complete the calculations, and the motors to move the optics to their required setting.

Before starting analysis, leave the spectrometer switched on for approximately 10 minutes to allow the lamps to warm up and stabilize.

2.1 Startup

1) Open the sample compartment cover.

2) Make sure that the beam paths are free, i.e.
   - No objects (cables etc.) project into the beam paths.
   - No samples are in the sample compartment.
   - Accessories are properly installed.

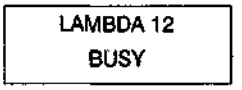
   Note: If the sample compartment is obstructed during the startup procedure, the spectrometer will not initialize correctly.

3) Close the sample compartment cover.

4) Switch on at the power switch.

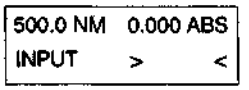
5) Wait for the standby display to appear.

   Lambda 14 or Lambda 14P shows on those spectrometers.



Initialization display

   The standby display. Other values may be shown.



Standby display

6) Switch on the accessories.

2.2 Shutdown

1) Return the spectrometer to standby, use **[STOP]** or **[PARAMETER]**.
2) Switch off the accessories.
3) Open the sample compartment cover.
4) Remove samples and cells from the sample compartment.
5) If accessories (e.g. flowcell) are installed in the sample compartment clean them thoroughly.
6) Close the sample compartment cover.
7) Switch off the spectrometer.

See also Wakeup, page 5-42.

2.3 Single Cell Holder

2.3.1 Description

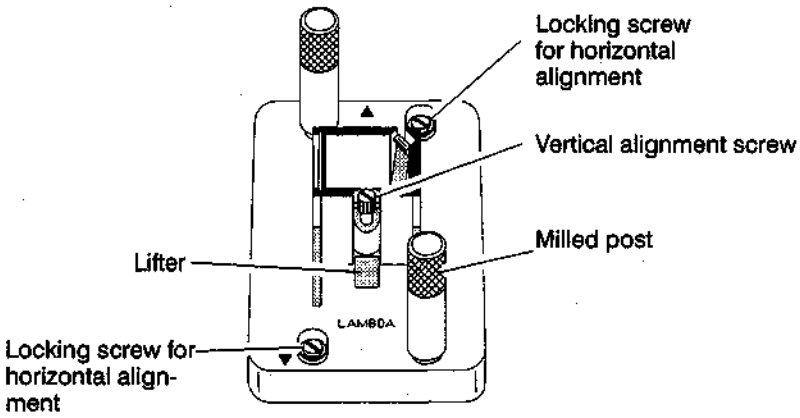


Figure 2-1 Single Cell Holder B050-5071

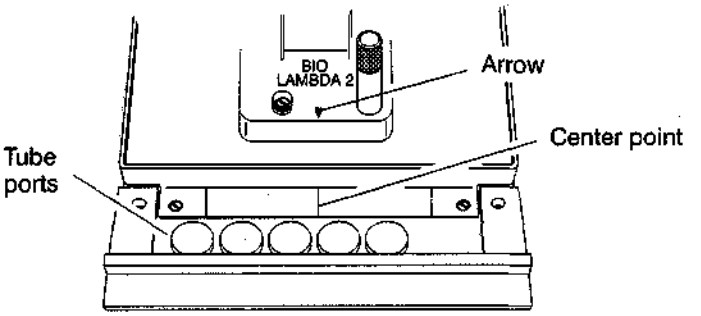
Note: *Depending on the spectrometer, the single cell holder can be installed in two different positions in the sample compartment. Always install the holder such that the arrow on the cell holder lines up with the center point on the baseplate (see next page):*

Inscription on Holder	***Use in Spectrometer***
LAMBDA *legible*	*In this position, the cell holder can be used with all Lambda Series Spectrometers.*
BIO LAMBDA 2 *legible*	*In this position, the cell holder can be used with Lambda 2 Series Spectrometers as Lambda 2, 11, 12, 14, 14P, Bio etc. (baseplate with 4 threaded holes). The smallest beam diameter is exactly in the middle of the cell. This is useful especially for operation with micro and semi-micro cells.*

2.3.2 Installing the Single Cell Holder

There are two single cell holders provided with the instrument, one for the sample beam and one for the reference beam. Install the single cell holder in the sample compartment as follows:

1) Lower the cell holder so that the two alignment holes slip onto the two studs on the baseplate at the bottom of the sample compartment.
The arrow on the cell holder must line up with the center point of the baseplate, and BIO LAMBDA 2 must be legible.



2) Move the milled posts a little to locate the threaded holes in the baseplate, and then tighten the milled posts.
The tube ports located at the front of the sample compartment allow you to lead tubes from flowcells, water-thermostatted cell holders, etc. in and out of the sample compartment.
When not in use, you should always insert the caps into the ports.

2.3.3 Aligning the Single Cell Holder

Coarse Alignment

1) Open the sample compartment cover.
Fill cells with a low-absorbing solvent (e.g., deionized water or ethanol).
Insert one cell into the sample cell holder and one into the reference cell holder.
Make certain that the cells are pushed down fully.
Note: The alignment procedure is for a given cell in a given holder. After alignment, the cell should always be used in the same holder.

2) Using the **[GOTOλ]** key, slew the monochromator to 0 nm to obtain a beam of visible (zero order) radiation in the sample compartment.

3) Block the sample and reference beam windows on the right-hand side of the sample compartment with a card to prevent white light from saturating the detector.

4) By holding a piece of matt white paper behind each cell holder, visually examine the light spot to see that the radiation beam is passing through the cell sample area. Diffraction patterns become apparent if the radiation beam impinges on the cell wall.

5) If the radiation beam is not centered exactly, loosen the two locking screws and the two milled posts on the relevant cell holder and shift the cell holder plate to center the radiation beam.
Then retighten the two milled posts and the two locking screws.

6) Now visually examine the vertical alignment of the radiation beam in the cell sample area.
Alignment is correct when the radiation beam is just above the floor of the cell sample area (min. 2 mm) or covers the cell window[1]:

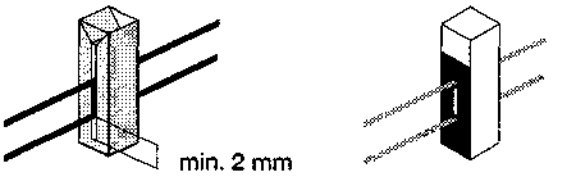


Figure 2-2 Correct Alignment of the Radiation Beam in the Cell Sample Area

7) If alignment is required, turn the vertical alignment screw on the lifter either clockwise to raise the cell, or counterclockwise to lower the cell.

8) Recheck the horizontal alignment of the radiation beam through the cell and correct if necessary.

9) Using the **[GOTOλ]** key, slew the monochromator to any value above 200 nm.

10) Remove the card blocking the sample and reference beam windows and close the sample compartment cover.

This completes coarse alignment of the cell holders.
If necessary, proceed with fine alignment as described on the next page.

1 The center of the window for micro flowcells should be ideally approximately 15 mm above the base of the cell.

Fine Alignment

If fine alignment is necessary, proceed as follows:

1) Using the **[GOTOλ]** key, slew the monochromator to the requested measurement wavelength or to 460 nm.

2) Call up a method that uses %T as the ordinate.
If necessary change the ordinate mode to %T.

3) Remove the reference cell from the sample compartment.

4) Make horizontal fine alignment to the sample cell holder (locking screws and milled posts loosened) to obtain the *highest* possible transmittance reading on the display (close the sample compartment cover while measuring transmittance).
Make fine alignment to the vertical alignment screw again to obtain the *highest* possible reading (close the sample compartment cover while measuring transmittance).

5) When you are satisfied with the alignment, tighten the milled posts and the locking screws on the cell holder.

6) Reinsert the reference cell in the reference cell holder.
The sample cell remains in its holder.

7) Repeat steps 4 and 5 with the reference cell holder, but this time obtain the *lowest* possible transmittance reading on the display.

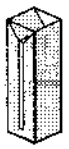
This completes the fine alignment procedure.

When the cell holder has been aligned once, you can take it out and reinstall it without aligning it again.

2.3.4 Minimum Volume Applications

To measure minimum sample volumes, use microcells (offered by Perkin-Elmer).
The minimum sample volume required is a function of the cell internal width or volume and is specified in Table 2-1.

Table 2-1 Minimum Volume Requirements

Cell Type		Cell Internal Width	Pathlength	Minimum Volume Required	Part Number
	Height of liquid slightly more than height of beam.	2 mm	1 cm	150 µL	B007-9404 (pair)
		4 mm	1 cm	300 µL	B007-9402 (pair)
		Cell Volume	**Pathlength**	**Minimum Volume Required**	**Part Number**
	Cell window completely filled with liquid.	0.5 µL	0.01 cm	2 µL	B051-0076
		2.5 µL	0.5 cm	5 µL	B051-0077
		5 µL	0.1 cm	10 µL	B051-0078
		5 µL	1.0 cm	10 µL	B050-5823
		30 µL	1.0 cm	50 µL	B019-0608

You should align microcells very carefully in the radiation beam by following the procedures in *Section 2.3.3*, above (*page 2-6*). When aligning microcells, fill each cell with the minimum volume of liquid specified in the above table to make sure that the liquid meniscus is not in the radiation beam.

Operating without Methods 3

Operating without Methods 3

3.1 Overview

Measurements are usually carried out using methods containing all the necessary parameters, see Chapter 5.

The following functions can be carried out via the keyboard:

- Setting the wavelength
- Manual background correction
- Quick sample measurement
- Reset

3.2 Setting the Wavelength Manually

The wavelength can be set manually using the **[GOTO λ]** key as follows:

...either...

1) Press **[GOTO λ]**.
2) Enter the desired wavelength, e.g. **325.5**.
3) Press **[ENTER]**.

   The monochromator slews to the selected wavelength.

...or...

1) Enter a wavelength using the numeric keys, e.g. **325.5**.
2) Then press **[GOTO λ]**.

   The monochromator slews to the selected wavelength.

3.3 Manual Background Correction

In this operation, the **[BACK CORR]** key is used to set the measured absorbance value to 0, or transmittance value to 100%, for the actual wavelength shown on the display.

1) Open the sample compartment cover.
2) Place cells with blank solution in the reference and sample cell holders.

   ...or...

   Place empty cells in the reference and sample cell holders.
3) Close the sample compartment cover.
4) Set the desired wavelength using **[GOTO λ]**.
5) Press **[BACK CORR]**.

Background correction is complete once the standby display reappears.

The ordinate mode of the last used method always appears on the display. To convert absorbance to transmittance or vice versa, select a TIME DRIVE method, and then select the desired ordinate mode.

3.4 Quick Sample Measurement

You can make quick sample measurements as follows:

1) Prepare the sample.
2) Press **[GOTO λ]**.
3) Select your desired wavelength.
4) Press **[ENTER]**.
   The monochromator slews to the selected wavelength.
5) Open the sample compartment cover.
6) Insert cells with blank solutions in the reference and sample cell holders.
   Or use air as blank.
7) Close the sample compartment cover.
8) Press **[BACK CORR]** and wait until the background correction is completed.
9) Open the sample compartment cover.
10) Remove the blank and insert the cell with sample solution in the sample cell holder.
11) Close the sample compartment cover.
12) The absorbance (*A*) or transmittance (%T) reading and wavelength are shown on the display.
    The ordinate mode of the last used method always appears on the display. Use a TIME DRIVE method to change from absorbance (A) to transmittance (%T).
13) Press **[0]** and then **[HELP]** to print out the reading.

3.5 Reset

By a full reset the spectrometer and its program are returned to the default condition.
You can carry out a full reset at any time.

Note: In carrying out a full reset, all methods will be erased. Before carrying out a full reset, make sure that all important methods are printed out.

To carry out a full reset:

1) Switch off the spectrometer.
2) Press **[7] [9] [•]** (seven, nine, point) simultaneously.
3) Keep the keys pressed and switch on the spectrometer.
4) Keep the keys pressed until the display appears.

After the full reset is completed a status report is printed out (when a printer is connected).

Note: There are default methods stored in the internal memory of the spectrometer. These methods are not deleted after a full reset and can be copied and amended (see Section 4.2.1, page 4-3).

How to use Methods 4

Methods 4

4.1 What are Methods?

Methods are a collection of those parameters necessary for a particular analysis using the spectrometer and are stored as method files.

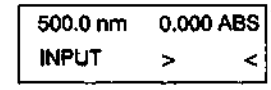
You can process large numbers of samples efficiently using the methods.
The parameter values necessary for the analysis in question need only be set once and are then available on request.

Up to 200 methods can be stored in the spectrometer; each method can be allocated a number between 1 and 999.

On delivery, 10 basic methods are programmed in the spectrometer; these are immediately available for use.

4.2 Selecting a Method

1) Switch to the standby display, use **[STOP]** or **[PARAMETER]**.

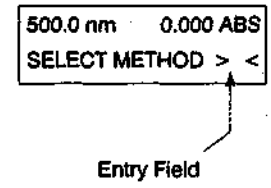


Standby display

2) Press **[METHOD]**.

3) Enter the method number.

4) Press **[ENTER]**.



The method is loaded onto the operational memory.

The method header then appears on the display.

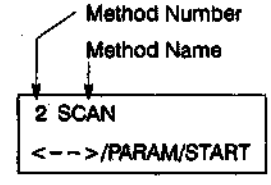
...or...

1) In the standby display, enter the method number.

2) Press **[METHOD]**.

The method is loaded onto the operational memory.

The method header then appears on the display.



Method header

The method can now be used for measurement.

If an unused method number is entered, the function NEW METHOD *appears.*
A new method can now be created (see Section 4.3.3, page 4-9).

If you don't know the method number, return to the standby display and use **[PARAMETER]** *to switch to the first method header. Then use the arrow keys to view the available methods in turn.*

4.2.1 Default Methods

Default methods are stored in the spectrometer. The default methods can be read and copied, but not modified.
The copied default methods can then be modified to suit your own requirements.

You access the default methods as follows:

1) Switch on the spectrometer in SuperUser mode (*see Appendix 1*).

2) Press **[STOP]** repeatedly until the APPLICATION branch header is displayed.

APPLICATION
PARAM/<– –>

3) Press **[•] [PARAMETER]** to select the first of the default methods.

4) Use the arrow key to select the required method type.

5) Press **[PARAMETER]** and then use the arrow key to select COPY FROM MARK.

6) Press **[STOP]** to return to the APPLICATION branch.

7) Create an empty method file (*see page 4-9*).

8) Copy the default method into the empty method file using the NEW FROM MARK parameter (*see page 4-14*).

The default method can now be amended as required.

4.3 Editing Methods

The following options are available:

- MODIFY METHOD
- DELETE METHOD
- NEW METHOD
- NEW METHOD NAME
- CHECK METHOD
- MARK FOR COPY, NEW FROM MARK
- PRINT METHOD

To recreate methods that have been inadvertently erased or written over, regularly print out all important methods.

4.3.1 Modifying a Method

1) Select the method to be modified.

2) Press **[PARAMETER]**.

3) Press **[PARAMETER]** again.

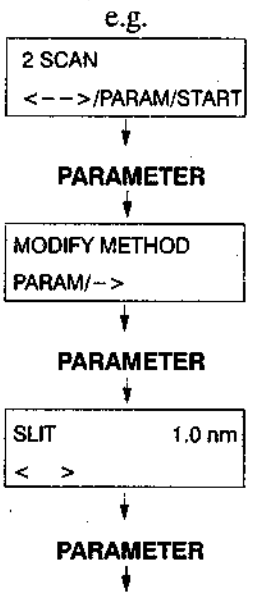
4) Change the displayed parameter values if required.

   ...or...
   Press **[PARAMETER]** to select the next parameter.

   ...or...
   Press **[–] [PARAMETER]** to recall the previous parameter.

   ...or...
   Enter the appropriate parameter number and press **[PARAMETER]** to select a particular parameter, *see Chapter 10* for parameter description and parameter numbers.

   ...or...
   Press **[STOP]** to cancel.



Changing a Parameter

1) Select the parameter to be changed.

2) Depending on the parameter shown, change as described in procedure table 4-1:

Table 4-1 Text and symbols on the lower line of the display.

Text/Symbol	Procedure
<– – >	Select option with the arrow keys.
ENTER:	Enter the desired value with the numeric keys. Press **[ENTER]** to confirm the value entered.
–>ENTER:	Appears if several values have to be entered. Use the arrow key to select the desired parameter. Enter the required value. Press **[ENTER]** to confirm the value entered. e.g. Several reference values have to be entered. Enter the value for **REF 1**. Confirm with **[ENTER]**. Use the arrow key to move to **REF 2**. Continue until values have been entered for all the references.

Press **[CE]** *to delete wrongly entered values.*

3) Press **[PARAMETER]** to proceed to the next parameter.

You can press **[HELP]** *for additional information about a parameter.*

When a particular parameter is set to YES *the extra parameters required automatically appear in their correct order.*
e.g.
When GRAPHICS PLOT *is* YES, *the extra parameters* ORD MAX, ORD MIN, SCALE *and* GRID *appear.*
When GRAPHICS PLOT *is* NO, *the extra parameters* ORD MAX, ORD MIN, SCALE *and* GRID *no longer appear.*

Tagging a Parameter

You tag a parameter to change it at appropriate times during the analysis, e.g. prior to the start of each sample measurement.

Untagged parameters can only be changed prior to the start of a method.

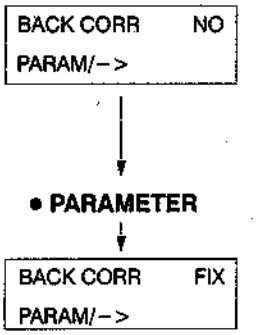
Table 4-2 shows the type of tagging, and when it appears during the analysis:

Table 4-2 Type of Tags

Tag	Symbol	Appears
CALL	&	Prior to the start of a method.
BATCH	!	Prior to the start of each sample batch.
START	*	Prior to the start of each sample.
FIX	FIX	No tag

Tag a parameter as follows:

1) Select the parameter to be tagged, for example:



2) Press **[•] [PARAMETER]**.

3) Select the appropriate tagging with the arrow keys.

4) Press **[ENTER]**.

Every parameter can be tagged. For parameters where tagging is less meaningful (e.g. LAMP, GRAPHICS PLOT*), tagging is accepted, but not carried out.*

4.3.2 Deleting a Method

1) Select a method that can be deleted.

2) Press **[PARAMETER]**.

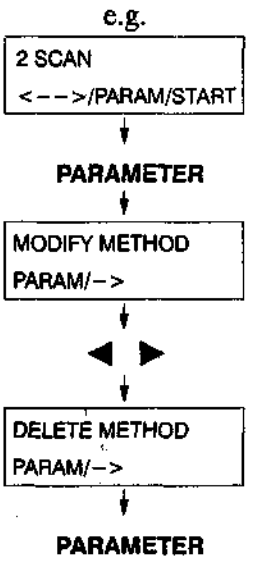
3) Use the arrow keys to select DELETE METHOD.

4) Press **[PARAMETER]** again to delete the method.

The method is deleted as soon as **[PARAMETER]** *is pressed, and the display returns to the next method header in the list.*

...or...

Press **[STOP]** to cancel.



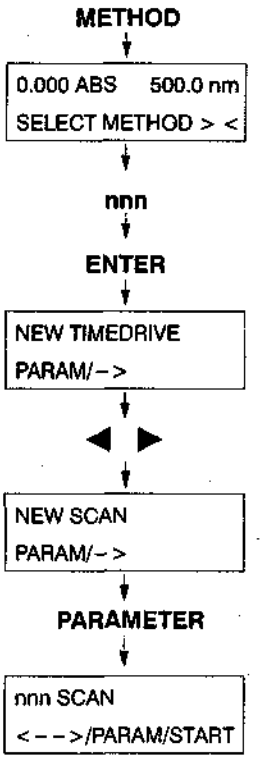
4.3.3 Creating a New Method

You can create a new method in one of the following ways:

1. Create a new empty method file.
2. Overwrite an existing method file

Creating an Empty Method File

1) Press **[METHOD]**.



2) Enter a method number **nnn** not previously used.
3) Press **[ENTER]**.

   The first method of the NEW METHOD level appears.
4) Use the arrow keys to select the required method type.
5) Press **[PARAMETER]**, this confirms the creation of the new method.
   ...or...
   Press **[STOP]** to cancel.
6) Modify the new method as required.

Overwriting an Existing Method

1) Select a method that can be overwritten.

2) Press **[PARAMETER]**.

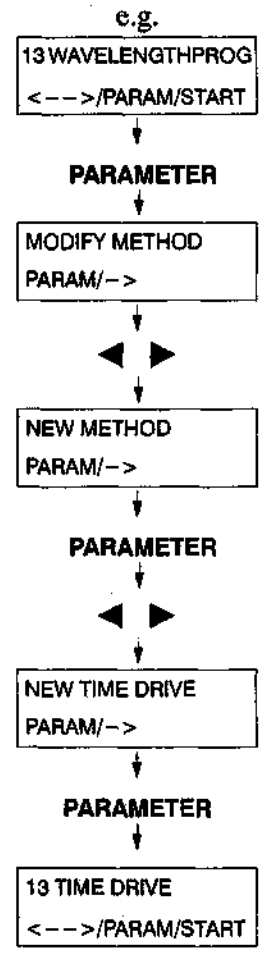
3) Use the arrow keys to select NEW METHOD.

4) Press **[PARAMETER]** again.

5) Use the arrow keys to select the method type.

6) Press **[PARAMETER]**.
The existing method is written over.
...or...
Press **[STOP]** to cancel.

7) Modify the new method as required.



4.3.4 New Method Name

You can rename methods using the NEW METHOD NAME parameter.

The method number remains the same when you rename a method.

1) Select the method to be renamed.

2) Press **[PARAMETER]**.

3) Use the arrow keys to select NEW METHOD NAME.

4) Press **[PARAMETER]** again.

5) Use the arrow keys to select letters. Confirm each letter by pressing **[ENTER]**.

   ...or...

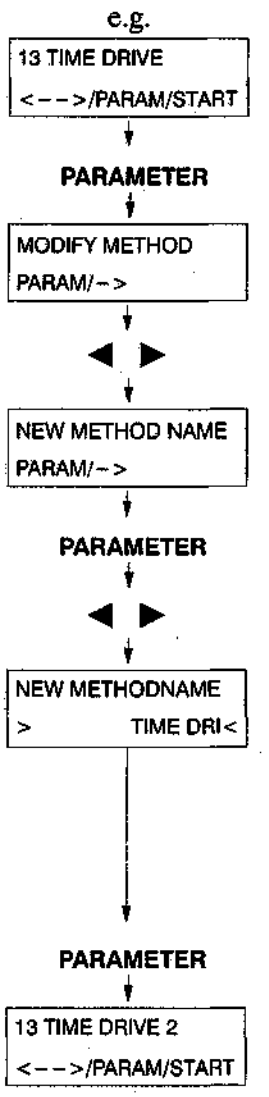
   Use the numeric keys to enter numbers. Confirm each number by pressing **[ENTER]**.

   ...or...

   Press **[ENTER]** without entering a letter or number for an empty space.

   You can combine numbers and letters.

6) Press **[PARAMETER]** to confirm the new name.



4.3.5 Checking a Method

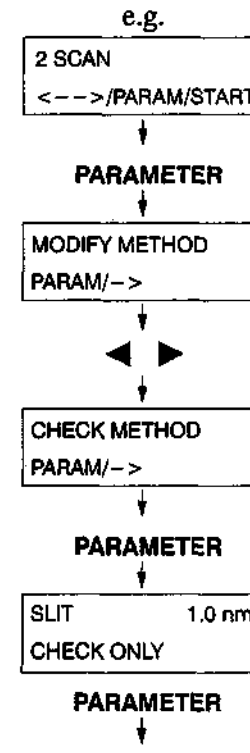
When using the CHECK METHOD function, the parameter values are displayed, but cannot be changed.

1) Select the method to be checked.

2) Press **[PARAMETER]**.

3) Use the arrow keys to select CHECK METHOD.

4) Press **[PARAMETER]** to check each parameter in turn.
...or...
Press **[STOP]** to cancel.



4.3.6 Copying Method Parameters into a New Method File

This is useful when you wish to make a new method with only a few parameters different from the original.
Two steps are involved in this procedure:

1. Marking the method for copy.
2. Copying the method into another method file.

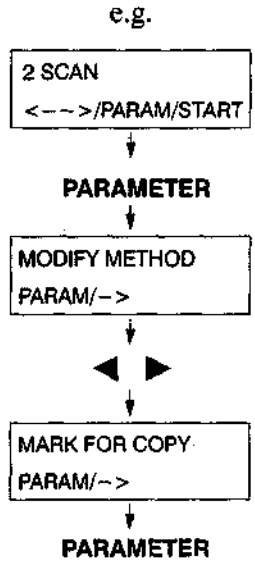
Marking for Copy

1) Select the method who's parameters are to be copied.

2) Press **[PARAMETER]**.

3) Use the arrow keys to select MARK FOR COPY.

4) Press **[PARAMETER]** to mark the method. The method is now marked for copying in the next step.
...or...
Press **[STOP]** to cancel.



Copying the Method into Another Method File

1) Create a method (*see Section 4.3.3, page 4-9)* into which the parameters from the marked method can be copied.

2) Press **[PARAMETER]**.

3) Use the arrow keys to select NEW METHOD.

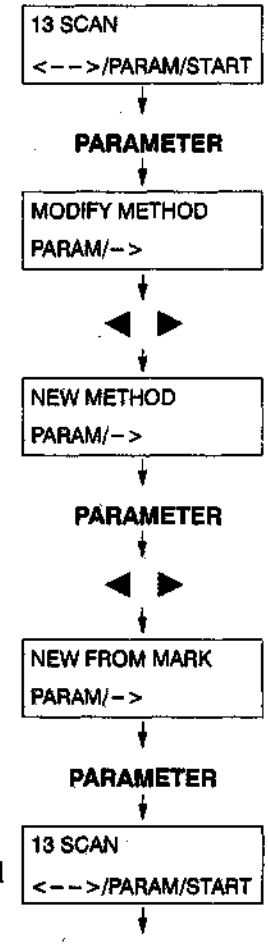
4) Press **[PARAMETER]** again.

5) Use the arrow keys to select NEW FROM MARK.

6) Press **[PARAMETER]** .

   The parameters from the marked method are copied into the newly created method.
   ...or...
   Press **[STOP]** to cancel.

7) Modify the new method as required.



4.3.7 Printing Out a Method

Printing out a method provides a list of its parameters and their current values.

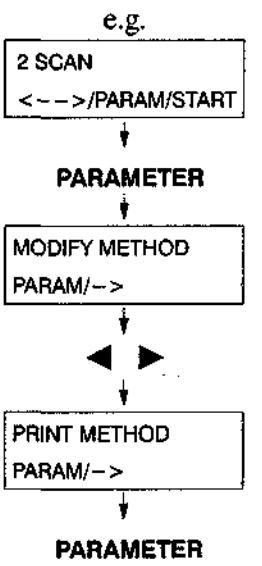
A printer must be installed and configured (refer to the installation handbook).

1) Select the method to be printed out.

2) Press **[PARAMETER]**.

3) Use the arrow keys to select PRINT METHOD.

4) Press **[PARAMETER]** again to print out the method.



...or...

Press **[STOP]** to cancel.

You can also press **[1]** *and then* **[HELP]** *to print out the method parameters.*

4.4 Printing Out a Directory

Printing out a directory provides a list of its methods.

A printer must be installed and configured (refer to the installation handbook).

There are two directories, the spectrometer directory and the branch directory.

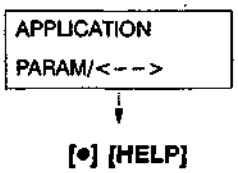
4.4.1 Spectrometer Directory

The spectrometer directory is a list of all methods for the spectrometer (including the SuperUser methods).
Print out the directory as follows:

1) Select a branch header.

2) Press [•] and then **[HELP]** to print out a directory of

e.g.



4.4.2 Branch Directory

The branch directory is a list of all the methods in the selected branch.
Print out all the methods in the selected branch as follows:

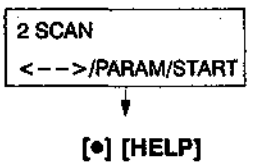
To select SuperUser branches, you must first enter as SuperUser, see Appendix 1.

1) Select a method in the branch to be printed out.

2) Press [•] and then **[HELP]** to print out the branch directory.

   You can also press **[2]** *and then* **[HELP]** *to print out the branch directory.*

e.g.



4.5 Help Key

The help key can be used on its own to provide additional information about the parameters currently shown on the display, or in combination with other keys to provide other functions, *see table 4-3*:

Table 4-3 Help Key Combinations

Key	Description
[HELP]	Provides additional parameter information on the display.
[•] [HELP]	Prints out a method or branch directory.
[0] [HELP]	Prints out the current values shown on the display.
[1] [HELP]	Prints out the method parameters.
[2] [HELP]	Prints out a directory of methods available in the branch.
[3] [HELP]	Prints out the additional method information.
[4] [HELP]	Line feed.
[5] [HELP]	Form feed.
[6] [HELP]	Prints out the Peltier cell holder temperature shown on the display. *Functions only when Peltier accessory is installed*
[7] [HELP]	Prints out spectrometer status.

To view the help text for a particular parameter proceed as follows:

1) Select the desired parameter.
2) Press **[HELP]**.
3) Continue to press **[HELP]** to view all the text.

   ...or...

   Press **[PARAMETER]** to interrupt the help function.

Help text is available in German, refer to the help configuration in SuperUser.

Measuring using Methods 5

Using Methods 5

5.1 Overview

The spectrometer incorporates the basic types of methods shown in the table below:

No.	Type of Method	Use	Section
1	TIME DRIVE	Measurement over a certain period at one wavelength.	5.4
2	SCAN	Scanning spectra and derivative spectra.	5.5
3	WAVELENGTH PROG	Measurement at several wavelengths; differential and ratio analysis at several wavelengths.	5.6
4	CONCENTRATION 1	Determination of concentration using peak height.	5.7.1
5	CONCENTRATION 2	Determination of concentration using peak area or 2nd derivative.	5.7.2
6	ENZYME KINETICS	Enzyme kinetics.	5.8
7	SUBSTRATE KIN	Substrate kinetics.	5.9
–	OLIGOQUANT 1	Quantitative analysis of oligonucleotides up to 50 bases long.	5.10
–	OLIGOQUANT 2	Quantitative analysis of oligonucleotides longer than 50 bases.	5.10
900	DATE/TIME	To enter and change the date and time.	5.11
901	WAKEUP	To switch on the lamps and allow them to warm up before the start of the working day.	5.12
999	SELF TEST	Instrument internal test to check the optics.	5.13

5.2 *Method Procedure*

When a method is selected, it can be used for measurements.

When starting the method, the system automatically makes requests via the display:

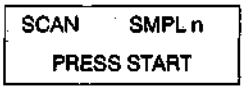
e.g.

Background correction:
Place a cell containing a blank solution in each of the sample and reference cell holders.

...or...

Place an empty cell in each of the sample and reference cell holders (measurement against air).

Press **[START]** to start the background correction.



Sample measurement:
Place the cell containing the sample solution in the sample cell holder.

n ENTER *can be used to switch directly to SAMPLE n; n is the sample number.*

Press **[START]** to start the measurement.

Tagged parameter (& is the CALL tag):
if desired, enter a new value and press **[ENTER]**.

...or...

Select a new value using the arrow keys.

Press **[START]** to proceed with the analysis.

5.3 Analysis Procedure

1) Select the appropriate method (*see Section 4.2, page 4-2*).
2) If necessary, modify the method parameters.
3) Press **[START]**.
4) Depending on the display:

SAMPLE ID ENTER > <	Change the displayed parameter values if required and press **[START]**.
... or...	
BACK CORR PRESS START	Insert cell(s) containing a blank solution and press **[START]**.
... or...	
XXXXXX SMPL 1 PRESS START	Insert a cell containing the sample solution and press **[START]**. *XXXX is any method type.*

5) Continue to insert samples when asked until they have all been measured.

Interrupting the Measurement

Press **[STOP]** to interrupt a method in progress.

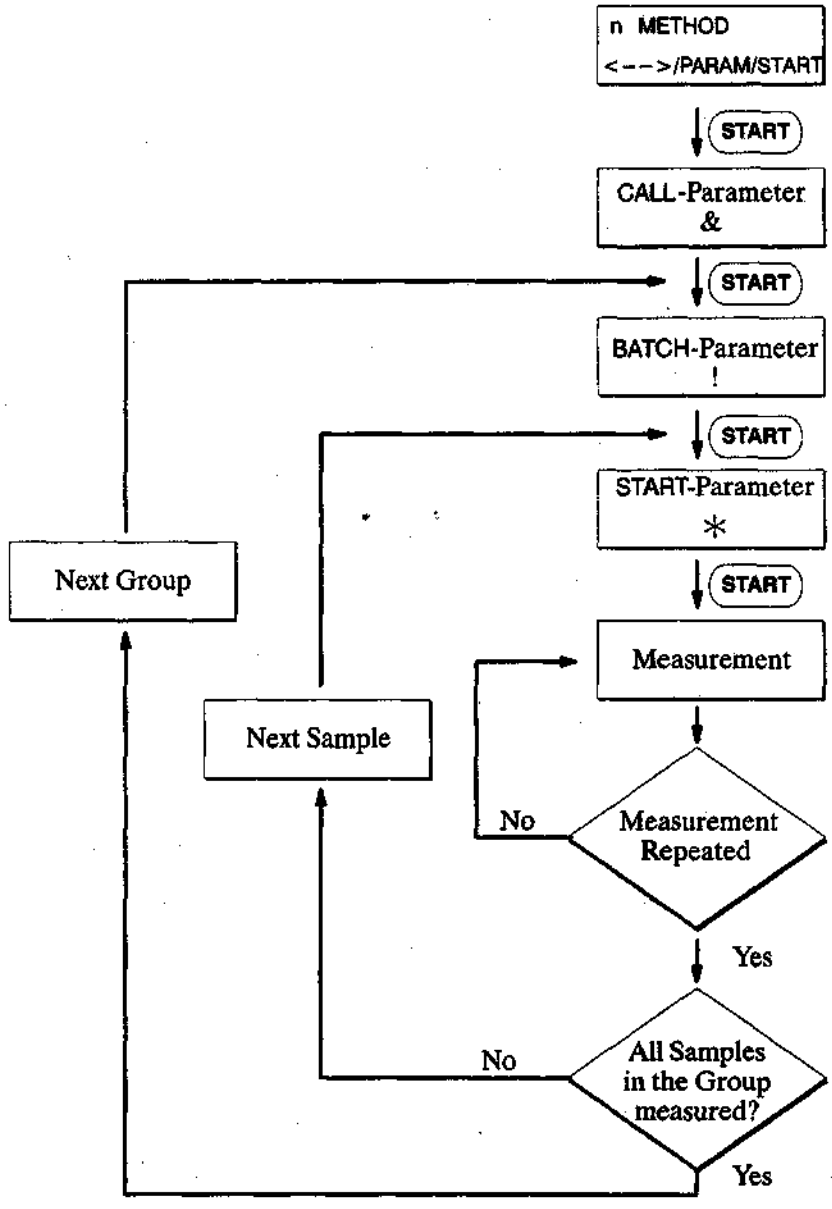


Figure 5-1 Flow diagram of a typical method procedure

5.4 Single Wavelength Measurements

Select a TIME DRIVE method to measure a sample at one wavelength over a defined period of time.

For Enzyme Activity methods, see Section 5.8, page 5-26.

Procedure

1) Select the desired TIME DRIVE method.

   The following table lists typical TIME DRIVE parameters in the order (left to right) in which they appear.
   See Section 10.1, page 10-1, for a detailed description of each parameter.

No.	Parameter	Value		No.	Parameter	Value
17	SLIT *	2.0 nm		1	ORDINATE MODE	ABS
3	WAVELENGTH	500.0 nm		11	FACTOR	1.0
14	RESPONSE	0.5 s		15	LAMP	UV + Vis
16	BACK CORR	YES		18	SAMPLES/BATCH	0
19	FIRST SAMPLE #	1		21	CYCLES	1
22	CYCLE–TIME	0.1 min		25	GRAPHICS PLOT	YES
26	ORD.MAX	0.000 ABS		27	ORD.MIN	1.000 ABS
28	SCALE	20 nm/min		29	GRID	NO
32	PRINT DATA	YES		35	AUTO METHOD	NO
36	OPER ID	.....		37	SAMPLE ID	.....

** Only available with Lambda 14 and Lambda 14P*

When a particular parameter is set to YES *the extra parameters required automatically appear in their correct order.*
e.g.
When GRAPHICS PLOT *is* YES, *the extra parameters* ORD MAX, ORD MIN, SCALE *and* GRID *appear.*
When GRAPHICS PLOT *is* NO, *the extra parameters* ORD MAX, ORD MIN, SCALE *and* GRID *no longer appear.*

2) If necessary, change the method parameters.

3) Press **[START]** to start the measurement.

4) Depending on the display:

SAMPLE ID ENTER > <	Change the displayed parameter values if required and press **[START]**.

... or...

BACK CORR PRESS START	Insert cell(s) containing a blank solution and press **[START]**.

... or...

TIMEDRIVE SMPL 1 PRESS START	Insert a cell containing the sample solution and press **[START]**.

5) Continue to insert samples when asked until they have all been measured.

Example of the Display Shown During the Measurement

xxx nm xxx min xxx ABS	xxx nm:	Wavelength.
	xxx ABS	Measured value; ordinate as selected.
	xxx min	Time; units as selected.
	C:xx	Repeat measurement cycles still to be performed This appears on the top right, when cycles >1.

Printout

The result is printed out at the end of the analysis.

5.5 Scanning a Spectrum

Select a SCAN method to scan and record a spectrum of the sample.

Procedure

1) Select the desired SCAN method.

   The following table lists typical SCAN parameters in the order (left to right) in which they appear.
   See Section 10.1, page 10-1, for a detailed description of each parameter.

No.	Parameter	Value	No.	Parameter	Value
17	SLIT*	2.0 nm	1	ORDINATE MODE	ABS
3	WAV. MAX	1100.0 nm	4	WAV MIN	190.0 nm
13	SPEED	960 nm/min	14	SMOOTH	2 nm
15	LAMP	UV+Vis	16	BACK CORR	YES
18	SAMPLES/BATCH	0	19	FIRST SAMPLE #	1
21	CYCLES	1	22	CYCLE TIME	0.1 min
25	GRAPHICS PLOT	YES	26	ORD.MAX	1.000 ABS
27	ORD.MIN	0.000 ABS	28	SCALE	50.0 nm/cm
29	GRID	YES	30	OVERLAY	NO
32	PRINT DATA	YES	33	THRESHOLD	0.1 ABS
35	AUTO METHOD	YES	36	OPER. ID	.....
37	SAMPLE ID	.....			

** Only available with Lambda 14 and Lambda 14P*

When a particular parameter is set to YES *the extra parameters required automatically appear in their correct order.*
e.g.
When GRAPHICS PLOT *is* YES, *the extra parameters* ORD MAX, ORD MIN, SCALE *and* GRID *appear.*
When GRAPHICS PLOT *is* NO, *the extra parameters* ORD MAX, ORD MIN, SCALE *and* GRID *no longer appear.*

2) If necessary, modify the method parameters.

3) Press **[START]** to start the method.

4) Depending on the display shown:

SAMPLE ID ENTER > <	Change the displayed parameter values if required and press **[START]**.

... or...

BACK CORR PRESS START	Insert cell(s) containing a blank solution and press **[START]**.

... or...

SCAN SMPL 1 PRESS START	Insert a cell containing the sample solution and press **[START]**.

5) Continue to insert samples when asked until they have all been measured.

Example of the Display Shown During the Measurement

SCAN SMPL 1 xxx nm xxx ABS	xxx nm:	Wavelength.
	xxx ABS	Measured value; ordinate as selected.
	CYC:xx	Repeat measurement cycles still to be performed This appears on the top right, when cycles >1.

Printout

Graphics are printed out during the measurement process; numerical data follow at the end of the analysis.

5.6 Measurement at Several Wavelengths

Select a wavelength program (WAVELENGTH PROG) method to measure a sample at several different wavelengths.

Procedure

1) Select the desired WAVELENGTH PROG method.

   The following table lists typical WAVELENGTH PROG parameters in the order (left to right) in which they appear.
   See Section 10.1, page 10-1, for a detailed description of each parameter.

No.	Parameter	Value		No.	Parameter	Value
17	SLIT*	2.0 nm		1	ORDINATE MODE	ABS
2	# WAVELENGTH	3		3	WAV. 1	459.9 nm
3	WAV. 2	418.5 nm		3	WAV. 3	360.0 nm
11	FACTOR 1	1.0		11	FACTOR 2	1.0
11	FACTOR 2	1.0		14	RESPONSE	0.5 S
15	LAMP	UV+Vis		16	BACK CORR	YES
18	SAMPLES/BATCH	0		19	FIRST SAMPLE #	1
21	CYCLES	1		22	CYCLE TIME	0.1 min
25	GRAPHICS PLOT	YES		26	ORD.MAX	1.000 ABS
27	ORD. MIN	0.000 ABS		28	SCALE	20 mm/min
29	GRID	YES		32	PRINT DATA	YES
35	AUTO METHOD	NO		36	OPER. ID	.....
37	SAMPLE ID	.....				

** Only available with Lambda 14 and Lambda 14P*

When a particular parameter is set to YES *the extra parameters required automatically appear in their correct order.*
e.g.
When GRAPHICS PLOT *is* YES, *the extra parameters* ORD MAX, ORD MIN, SCALE *and* GRID *appear.*
When GRAPHICS PLOT *is* NO, *the extra parameters* ORD MAX, ORD MIN, SCALE *and* GRID *no longer appear.*

2) If necessary, modify the method parameters.

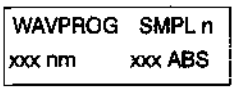
3) Press **[START]** to start the measurement.

4) Depending on the display:

SAMPLE ID ENTER > <	Change the displayed parameter values if required and press **[START]**.
... or...	
BACK CORR PRESS START	Insert cell(s) containing a blank solution and press **[START]**.
... or...	
WAVPROG SMPL 1 PRESS START	Insert a cell containing the sample solution and press **[START]**.

5) Continue to insert samples when asked until they have all been measured.

Example of the Display Shown During the Measurement



xxx nm:	Wavelength.
xxx ABS	Measured value; ordinate as selected.
CYC:xx	Repeat measurement cycles still to be performed This appears on the top right, when cycles >1.

Printout

The result is printed out at the end of the analysis.

5.7 Concentration Determination

You use CONCENTRATION 1 and CONCENTRATION 2 methods to determine the sample concentration.

Using CONCENTRATION methods, you first establish a calibration curve and then measure the sample concentration.

The instrument calculates the calibration curve from the corrected or uncorrected values at defined wavelengths via the peak heights (CONCENTRATION 1), or the peak areas (CONCENTRATION 2), or the 2nd derivative (CONCENTRATION 2) of the spectrum.

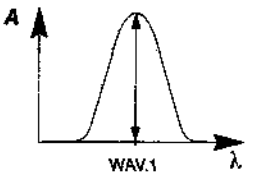
5.7.1 CONCENTRATION 1 Method (Peak heights)

Summary of the procedure for creating a CONCENTRATION 1 method:

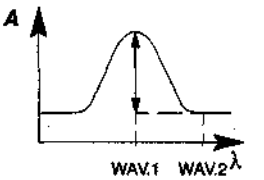
- Determine the measurement wavelength(s) (*see page 5-13*).
- Create a CONCENTRATION 1 method (*see page 5-14*).
- Establish a calibration curve using references (*see page 5-15*).
- Measure the sample (*see page 5-16*).

Determining the Measurement Wavelength(s)

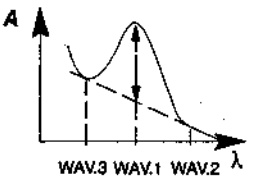
1) To determine the wavelengths:
   a) Record the spectrum of the sample (*see* SCAN *method, page 5-8*).
   b) Select a strong peak and note the wavelength at its absorbance maximum (WAV. 1).



2) Select the type of baseline correction required:
   a) With a straight but offset baseline: Select a second wavelength at the baseline minimum (WAV. 2).



   b) With a sloping baseline: Select a wavelength at the beginning and at the end of a peak (WAV. 2 and WAV. 3).



Creating a Method

1) Create a new CONCENTRATION 1 method (*see Section 4.3.3, page 4-9*).

   The following table lists typical CONCENTRATION 1 parameters in the order (left to right) in which they appear. *See Section 10.1, page 10-1, for a detailed description of each parameter.*

No.	Parameter	Value		No.	Parameter	Value
17	SLIT*	2.0 nm		1	MODE	ABS
3	WAV. 1	3		2	# OF REFS	3
6	CONC UNIT	µg/mL		7	REF 1	1.0 µg/mL
7	REF 2	2.0 µg/mL		7	REF 3	3.0 µg/mL
9	REFS.	NEW		8	VALUE 1	0.1
8	VALUE 2	0.2		8	VALUE 3	0.3
10	CUR FIT	LINEAR		11	FACTOR	1.0
12	DIVISOR	1.0		14	RESPONSE	1 s
15	LAMP	UV+Vis		16	BACK CORR	YES
18	SAMPLES/BATCH	0		19	FIRST SAMPLE	1
21	CYCLES	1		22	CYCLE–TIME	0.1 min
32	PRINT DATA	YES		25	PLOT REFS	YES
26	PRINT REFS	YES		35	AUTO METHOD	YES
36	OPER. ID	.....		37	SAMPLE ID	.....

** Only available with Lambda 14 and Lambda 14P*

2) Modify the parameters as required, using the wavelengths determined above.

   Once a method is created you can save it and use it for the same analysis when required without having to redetermine the wavelengths.

Establishing the Calibration Curve

1) Press **[START]** to start the measurement.

2) Depending on the display:

SAMPLE ID ENTER > <	Change the displayed parameter values if required and press **[START]**.

...or...

BACK CORR PRESS START	Insert cell(s) containing a blank solution and press **[START]**.

...or...

REF n [xxx] PRESS START	Insert a cell containing a reference solution and press **[START]**.

3) Insert the references in sequence when asked.
When they have all been measured, the instrument prints out the calibration curve and results.

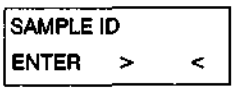
You can now amend the calibration curve (*see Section 5.7.3 page 5-24*) if required.

You can use previously established calibration curves, or reference values (see REFS, *page 10-21).*

Measuring the Sample

1) Press **[START]** to start the measurement.

2) Depending on the display:

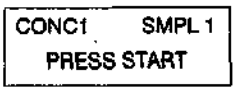


Change the displayed parameter values if required and press **[START]**.

... or...

Insert cell(s) containing a blank solution and press **[START]**.

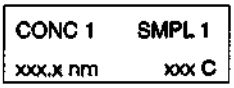
... or...



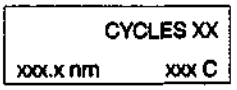
Insert a cell containing the sample solution and press **[START]**.

3) Continue to insert samples when asked until they have all been measured.

Example of the Display Shown During the Measurement



xxx nm: Wavelength.
xxx C: Result; units as selected.



CYCLES:XX Repeat measurement cycles still to be performed
This appears on the top right,when cycles >1.

Printout

If PLOT REFERENCES and PRINT DATA are set to YES, the calibration curve and results are printed out.

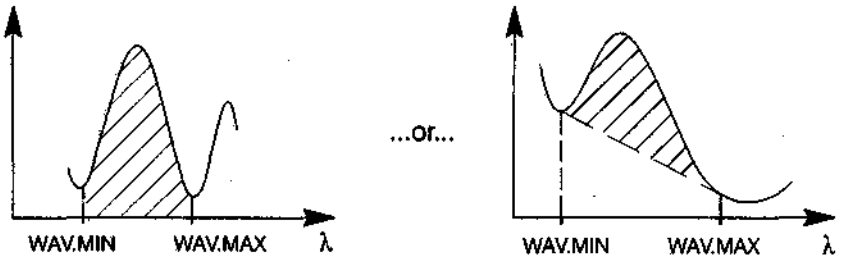
5.7.2 CONCENTRATION 2 Method (Peak Areas, 2nd Derivative)

Summary of procedure for creating a CONCENTRATION 2 method:

- Determine the measurement wavelengths (*see page 5-19*).
- Determine the threshold value (2nd derivative) (*see page 5-20*).
- Create a CONCENTRATION 2 method (*see page 5-21*).
- Establish a calibration curve using references (*see page 5-22*).
- Measure the sample (*see page 5-23*).

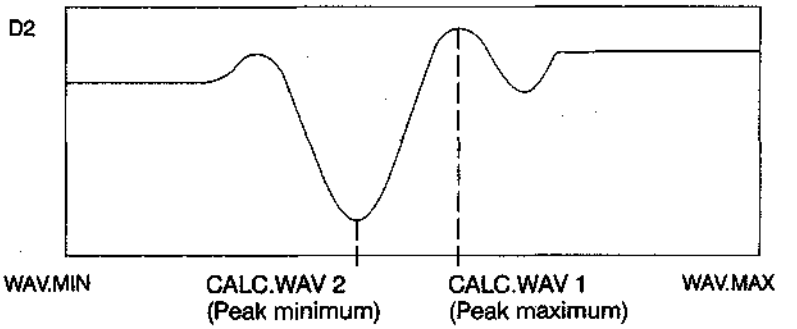
Determining the Measurement Wavelengths (Peak areas)

1) To determine the wavelengths:
   a) Record the spectrum of the sample (*see* SCAN *method, page 5-8*).
   b) Select a strong peak and note the wavelength at its start (WAV. MAX) and end (WAV. MIN).



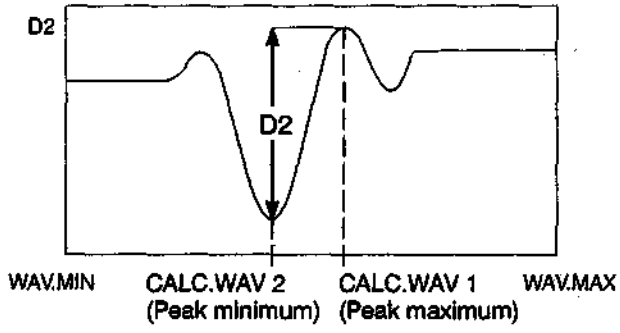
Determining the Measurement Wavelengths (2nd derivative)

1) To determine the wavelengths:
   a) Record the spectrum of the sample (*see* SCAN *method, page 5-8*).
   b) Select a strong peak and note the wavelength at its start (WAV. MAX) and end (WAV. MIN).
   c) Record the spectrum of the same sample using the 2nd derivative (D2 mode) over the wavelength range determined in step b above.
   d) From the 2nd derivative spectrum determine the wavelength at the peak maximum and peak minimum. Use these values for CALC.WAV 1 and CALC. WAV 2.



Determining the Threshold Value (2nd derivative)

1) To determine the threshold value:
   a) Record the spectrum of the most dilute reference solution using the 2nd derivative (D2 mode) over the wavelength range determined in step 1b of *Determining the measurement wavelengths (2nd derivative)* above.
   b) Determine the value D2 of this spectrum, i.e. the height from peak maximum to peak minimum.



   c) Select a value for the threshold parameter somewhat lower than this D2 value.

Creating a Method

1) Create a new CONCENTRATION 2 method (*see Section 4.3.3, page 4-9*).

   The following table lists typical CONCENTRATION 2 parameters in the order (left to right) in which they appear. *See Section 10.1, page 10-1, for a detailed description of each parameter.*

No.	Parameter	Value		No.	Parameter	Value
17	SLIT*	2.0 nm		1	MODE	PEAK AREA
3	WAV. MAX	600.0 nm		4	WAV. MIN	500.0 nm
2	# OF REFS	3		6	CONC UNIT	C
7	REF 1	1.0 C		7	REF 2	2.0 C
7	REF 3	3.0 C		9	REFS	NEW
8	VALUE 1	0.1		8	VALUE 2	0.2
8	VALUE 3	0.3		10	CUR FIT	LINEAR
11	FACTOR	1.0		12	DIVISOR	1.0
13	SPEED	960 nm/min		14	SMOOTH	2 nm
15	LAMP	UV+Vis		16	BACK CORR	YES
18	SAMPLES/BATCH	0		19	FIRST SAMPLE	1
21	CYCLES	1		22	CYCLE–TIME	0.1 min
32	PRINT DATA	YES		25	PLOT REFS	YES
26	PRINT REFS	YES		35	AUTO METHOD	YES
36	OPER. ID	.....		37	SAMPLE ID	.....

** Only available with Lambda 14 and Lambda 14P*

2) Modify the parameters as required, using the values determined above.

   Once a method is created you can save it and use it for the same analysis when required without having to redetermine the values.

Establishing the Calibration Curve

1) Press **[START]** to start the measurement.

2) Depending on the display:

SAMPLE ID ENTER > <	Change the displayed parameter values if required and press **[START]**.
... or...	
BACK CORR PRESS START	Insert cell(s) containing a blank solution and press **[START]**.
... or...	
REF n [XXX] PRESS START	Insert a cell containing the sample solution and press **[START]**.

3) Insert references in sequence when asked.
When they have all been measured, the instrument prints out the calibration curve and results.

You can now amend the calibration curve (*see Section 5.7.3, page 5-24*) if required.

You can use previously established calibration curves, or reference values (see **REFS**, *page 10-21).*

Measuring the Sample

1) Press **[START]** to start the measurement.

2) Depending on the display:

SAMPLE ID ENTER > <	Change the displayed parameter values if required and press **[START]**.

... or...

BACK CORR PRESS START	Insert cell(s) containing a blank solution and press **[START]**.

... or...

CONC2 SMPL 1 PRESS START	Insert a cell containing the sample solution and press **[START]**.

3) Continue to insert samples when asked until they have all been measured.

Example of the Display Shown During the Measurement

CONC 2 SMPL 1 xxx.x nm xxx C	xxx nm: xxx C:	Wavelength. Result; units as selected.

CYCLES XX xxx.x nm xxx C	CYCLES:XXRepeat measurement cycles still to be performed This appears on the top right,when cycles >1.

Printout

If PLOT REFERENCES and PRINT DATA are set to YES, the calibration curve and results are printed out.

5.7.3 Processing the Calibration Curve (CONCENTRATION Methods)

Changing the Type of Curve Fit

The type of calibration curve fit (linear or quadratic) can be altered without having to carry out additional measurements. The procedure is as follows:

1) Modify the method: Select REFS = OLD
   CUR FIT as required

2) Press **[START]**.

The new curve fit is calculated on already available data.

Remeasuring the Reference Solution

Should a measuring point lie outside the calibration curve and need to be remeasured, proceed as follows:

1) Modify the method: select REFS = NEW.

2) Press **[START]**.

3) When asked for first reference solution, enter the number of the reference to be remeasured and press **[ENTER]**.

REF 1 [xxx]
PRESS START

4) Place the reference solution in the sample cell holder and press **[START]**.

5) Wait until measurement is complete and then press **[STOP]**.

6) Set REFS = OLD.

7) Press **[START]**.

   The new calibration curve is calculated with the new value.
   If the new curve is satisfactory, measure the sample,
   ...or...
   if it is not acceptable, repeat the procedure.

Deleting a Point from the Calibration Curve

To delete such a point proceed as follows:

Linear curve through zero

In the method parameters;

1) Set REF n = 0.000 and VALUE n = 0.000.
   n is the number of reference solutions.

2) Press **[START]** to confirm the changes.

   ...or...

   Press **[STOP]** to cancel.

Non linear curves, and curves with intercept

In the method parameters;

1) Note the ordinate value and the concentration value of the last point.

2) Replace the ordinate value and the concentration value of the point to be deleted (REF n) with the values noted in step 1 above.

2) Reduce the value for # OF REFS by 1.

3) Press **[START]** to confirm the changes.

   ...or...

   Press **[STOP]** to cancel.

5.8 Enzyme Kinetics

Select an ENZYME method for enzyme kinetic measurements.

Note: Enzyme activity is strongly dependent on temperature. Thus, the following should be taken into account:

- *All measurements should be carried out at a constant temperature. You can use the temperature sensor (Part Number B018-5227) for monitoring the temperature.*
- *All solutions and essential instrument accessories, especially cells and cell holders, should be thermostatted prior to use.*

Procedure

1) Select the appropriate ENZYME method.

   The following table lists typical ENZYME parameters in the order (left to right) in which they appear.
   See Section 10.1, page 10-1, for a detailed description of each parameter.

No.	Parameter	Value		No.	Parameter	Value
17	SLIT*	2.0 nm		3	WAVELENGTH	340.0 nm
14	RESPONSE	0.5 s		15	LAMP	UV+Vis
20	TIME UNIT	min		9	CALCULATE	REGRESSION
22	TOTAL TIME	1.0 min		21	INTERVAL	0.2 min
24	LAG TIME	0.0 min		11	ENZ.FACTOR	1.0
10	DIL. FACTOR	1.0		12	DIVISOR	1.0
7	BLANK	0.0		6	ENZ.UNITS	U/L
16	BACK CORR	YES		14	SAMPLES/BATCH	0
19	FIRST SAMPLE #	1		25	GRAPHICS PLOT	YES
26	ORD.MAX	1.000 ABS		27	ORD.MIN	0.000 ABS
28	SCALE	20 mm/min		29	GRID	YES
32	PRINT DATA	ALL		34	POSTRUN KIN.	YES
35	AUTO METHOD	YES		36	OPER. ID	.....
37	SAMPLE ID	.....				

** Only available with Lambda 14 and Lambda 14P*

2) Modify the method as required.

3) If necessary, determine the blank value of the reaction (*see page 5-28*) and enter the value in the parameter BLANK.

4) Press **[START]** to start the measurement.

5) Depending on the display shown:

SAMPLE ID ENTER > <	Change the displayed parameter values if required and press **[START]**.
...or...	
n BACK CORR PRESS START	Insert the cell containing a blank solution (distilled water) and press **[START]**.
...or...	
n SAMPLE 1 PRESS START	Place solutions in the cell according to the procedure and mix. Place the cell in the sample cell holder. Press **[START]** to start the measurement or to continue the method.

Example of the Display Shown During the Measurement

xxx nm xxx min xxx ABS	xxx nm: Wavelength. xxx min: Time. xxx ABS Measured value.

The display shown when using a cell changer:

EN x°C n C:001 xxx min xxx ABS	EN: Type of method. x°C: Temperature. n: Cell location. C:001: Cycle number. xxx min: Interval time. xxx ABS Measured value.

Printout

The results are printed out at the end of the analysis.

Determining the Blank Value

Determine the blank value of the reaction as follows:

1) Select the appropriate ENZYME method.
2) Set parameter BLANK = 0.0 in the method.
3) Carry out a measurement according to your procedure using a cell filled with redistilled water in place of the sample.
4) Enter the result of the measurement in the parameter BLANK.

5.9 Substrate Kinetics

Select a SUBSTRATE KIN method for substrate kinetic measurements.

Manual Procedure

1) Select the appropriate SUBSTRATE KIN method.

   The following table lists typical SUBSTRATE KIN parameters in the order (left to right) in which they appear.
   See Section 10.1, page 10-1, for a detailed description of each parameter.

No.	Parameter	Value		No.	Parameter	Value
17	SLIT*	2.0 nm		3	WAVELENGTH	340.0 nm
14	RESPONSE	0.5 s		15	LAMP	UV+Vis
20	TIME UNIT	min		23	DELAY TIME	0.0 min
22	END TIME	3.0 min		21	CREEPING CYCLE	0
11	CONC FACTOR	1.0		10	DIL.FACTOR	1.0
12	DIVISOR	1.0		7	BLANK	0.0
6	CONC UNIT	C		16	BACK CORR	YES
18	SAMPLES/BATCH	0		19	FIRST SAMPLE #	1
25	GRAPHICS PLOT	YES		26	ORD.MAX	1.000 ABS
27	ORD.MIN	0.000 ABS		28	SCALE	21 mm/min
29	GRID	YES		32	PRINT DATA	ALL
34	POSTRUN KIN	YES		35	AUTO METHOD	YES
36	OPER. ID	.....		37	SAMPLE ID	.....

** Only available with Lambda 14 and Lambda 14P.*

2) Modify the method as required.

3) If necessary, determine the blank value of the reaction (*see page 5-33*) and enter the value in the parameter BLANK.

4) Press **[START]** to start the measurement.

5) Depending on the display shown:

SAMPLE ID ENTER > <	Change the displayed parameter values if required and press **[START]**.
...or...	
n BACK CORR PRESS START	Insert the cell containing a blank solution (distilled water) and press **[START]**.
...or...	
n SAMPLE 1 PRESS START	Place solutions (with the exception of enzyme solution) in a cell and mix. Place the cell in the sample cell holder and press **[START]**.

6) Allow equilibrium time (delay time) to elapse.

DELAY TIME xxx nm xxx MIN xxx ABS

7) Add the enzyme solution, mix and press **[START]**.

–WAIT– SAMPLE 1 PRESS START

Procedure with a Cell Changer

Analysis is performed analogous to manual operation, the essential difference being that instead of one cell several can be used for measurement in the one operation.

The procedure is as follows:

1. Once the equilibrium time has elapsed (prior to addition of enzyme), the absorbance in each cell is measured automatically.
2. After adding enzyme, only location 1 is measured to follow the course of the reaction.
3. Once the reaction is complete the absorbance in all the remaining cells is measured.

Example of the Display Shown During the Measurement

SUBSTRATE xxx nm xxx min xxx ABS	SUBSTRATE: Type of method. xxx nm: Wavelength. xxx min: Time. xxx ABS: Measured value.

The display shown when using a cell changer:

SUBST x°C n xxx min xxx ABS	SUBST: Type of method. x°C: Temperature. n: Cell location. xxx min: Time. xxx ABS: Measured value.

Printout

The results are printed out at the end of the analysis.

Determining the Blank Value

Determine the blank value of the reaction as follows:

1) Select the appropriate SUBSTRATE method.
2) Set parameter BLANK = 0.0 in the method.
3) Carry out a measurement according to your procedure using a cell filled with redistilled water in place of the sample.
4) Enter the result of the measurement in the parameter BLANK.

5.9.1 Recalculation of Results with POSTRUN KIN

You can recalculate the results from ENZYME and SUBSTRATE methods using the POSTRUN KIN parameter.

When you set the POSTRUN KIN parameter to YES the following parameters in the corresponding method can be modified:

ENZYME method: LAG TIME and TOTAL TIME
SUBSTRATE method: END TIME (manual operation only)

Note: Observe the following:
The time set for the LAG TIME *must be smaller than the* TOTAL TIME.
If a cell changer is being used, make sure that both LAG TIME *and* TOTAL TIME *are whole multiples of the* INTERVAL *time.*

Proceed as follows to calculate the results:

1) Create a method with POSTRUN KIN = YES.

2) Carry out a measurement (*see Section 5.8 or 5.9*).

   At the end of the measurement POSTRUN KIN appears on the display.

3) Press **[START]** to recalculate results.

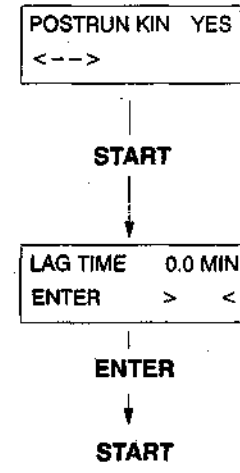
   ...or...

   If the results are not to be recalculated, select NO using the arrow keys and press **[START]**.

4) Enter new values as required for the parameter displayed and press **[ENTER]**.

5) Press **[START]** to continue.

e.g.



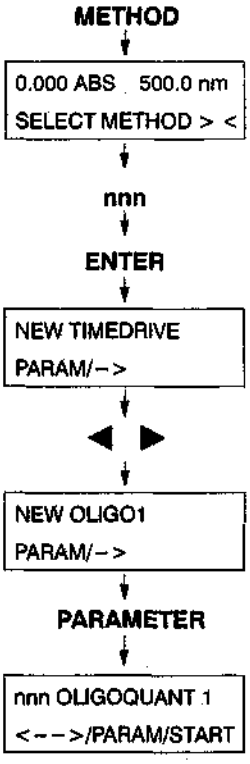
5.10 Methods for Quantitative Analysis of Oligonucleotides

Select an OLIGOQUANT 1 method for quantitative analysis of oligonucleotides up to 50 bases long, and to calculate the theoretical melting point.

Select an OLIGOQUANT 2 method for quantitative analysis of oligonucleotides longer than 50 bases, and to calculate the theoretical melting point.

You create an OLIGOQUANT method as follows:

1) Press **[METHOD]**.



2) Enter a method number **nnn** not previously used.

3) Press **[ENTER]**.

   The first method of the NEW METHOD level appears.

4) Use the arrow keys to select the required method type (Oligoquant 1 or Oligoquant 2).

5) Press **[PARAMETER]**, this confirms the creation of the new method.
   ...or...
   Press **[STOP]** to cancel.

6) Modify the new method as required.

Procedure

1) Select the appropriate OLIGOQUANT method (*see page 5-38*).
2) If necessary, modify the method parameters.
3) Press **[START]** to start the measurement.
4) Depending on the display:

SAMPLE ID ENTER > <	Change the displayed parameter values if required and press **[START]**.
... or...	
BACK CORR PRESS START	Insert cell(s) containing a blank solution and press **[START]**.
... or...	
OLIGO n SMPL 1 PRESS START	Insert a cell containing the sample solution and press **[START]**.

5) Continue to insert samples when asked until they have all been measured.

Example of the Display Shown During the Measurement

OLIGO n	SMPL n	xxx nm:	Wavelength.
xxx nm	xxx ABS	xxx ABS	Measured value; ordinate as selected.

Printout

Graphics are printed out during the measurement process; numerical data follow at the end of the analysis.

Oligoquant Parameter Tables

The following table lists typical OLIGOQUANT 1 parameters in the order (left to right) in which they appear.
See Section 10.1, page 10-1, for a detailed description of each parameter.

No.	Parameter	Value	No.	Parameter	Value
17	SLIT*	2.0 nm†	1	ORDINATE MODE	ABS†
2	# WAVELENGTHS	1 †	3	WAV. 1	260.0 nm†
11	FACTOR 1	1.0	59	PATHLENGTH	1.0 cm
60	SEQUENCE LENGTH	20	61	SEQ.	1
61	SEQ	2	65	CHNGE CONSTANTS	NO
76	TM CALCULATION	NO	14	RESPONSE	1 s
15	LAMP	UV+Vis	16	BACK CORR	NO
18	SAMPLES/BATCH	0	19	FIRST SAMPLE #	1
21	CYCLES	1	22	CYCLE TIME	0.01 min
25	GRAPHICS/PLOT	NO†	32	PRINT DATA	YES
35	AUTO METHOD	NO	36	OPER. ID	.....
37	SAMPLE ID	.....			

** Only available with Lambda 14 and Lambda 14P.*
†Do not change the value for this parameter.

Once the OLIGOQUANT 1 method is created, the method parameters can be edited.
The base sequence must be entered from base 5′ to 3′.
Use the numeric keys according to the table below to enter the base sequence.

Base	Description	Key
N	Any base	8
A	Adenine	7
C	Cytosine	4
G	Guanine	1
T	Thymine	0

The following table lists typical OLIGOQUANT 2 parameters in the order (left to right) in which they appear.
See Section 10.1, page 10-1, for a detailed description of each parameter.

No.	Parameter	Value		No.	Parameter	Value
17	SLIT*	2.0 nm		1	ORDINATE MODE	ABS†
2	# WAVELENGTH	1†		3	WAV. 1	260.0 nm†
11	FACTOR 1	1.0		59	PATHLENGTH	1.0 cm
60	NUMBER OF dA	0		60	NUMBER OF dC	0
60	NUMBER OF dG	0		60	NUMBER OF dT	0
60	NUMBER OF N	0		65	CHNGE CONSTANTS	NO
76	TM CALCULATION	NO		14	RESPONSE	0.5 S
15	LAMP	UV+Vis		16	BACK CORR	YES
18	SAMPLES/BATCH	0		19	FIRST SAMPLE #	1
21	CYCLES	1		22	CYCLE TIME	0.1 min
25	GRAPHICS PLOT	NO		32	PRINT DATA	YES
35	AUTO METHOD	NO		36	OPER. ID	.....
37	SAMPLE ID	.....				

** Only available with Lambda 14 and Lambda 14P.*
† Do not change the value for this parameter.

5.11 Date/Time

1) Select the DATE/TIME method (900).

2) Press **[PARAMETER]**.

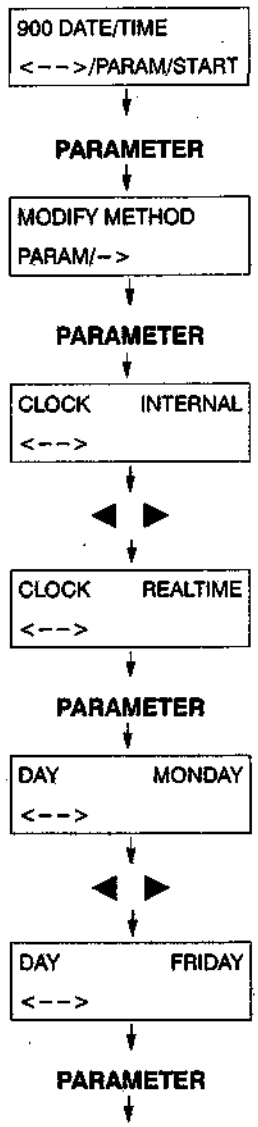
3) Press **[PARAMETER]** again.

4) Use the arrow keys to select the realtime or the internal clock.

5) Press **[PARAMETER]** again.

6) Use the arrow keys to select the day.

7) Press **[PARAMETER]** again.



8) Type in the date using the numeric keys; year, month day. e.g. 940429.

9) Press **[ENTER]** to confirm the entry.

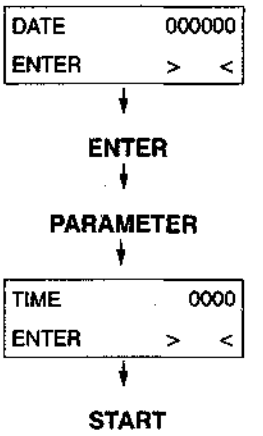
10) Press **[PARAMETER]** again.

11) Type in the time using the numeric keys; hours, minutes, e.g. 1430.

12) Press **[START]** to activate the clock.

...or...

Press **[STOP]** to cancel.



The realtime clock need only be set once, and has the following functions: day (Monday, Tuesday, etc...), date (yymmdd) and time (hhmm), and continues working when the instrument is switched off.

The internal clock is limited to the following functions: date (yymmdd) and time (hhmm), and counts from the time the spectrometer is switched on.
The internal clock must be reset to actual time after each switch on.

5.12 Wakeup

You can use WAKEUP to set the spectrometer to switch on the lamps to warm up before the start of the working day.

1) Select the WAKEUP method (901).

2) Press **[PARAMETER]**.

3) Press **[PARAMETER]** again.

4) Type in the 'wakeup' date using the numeric keys; year, month, day. e.g. 940429.

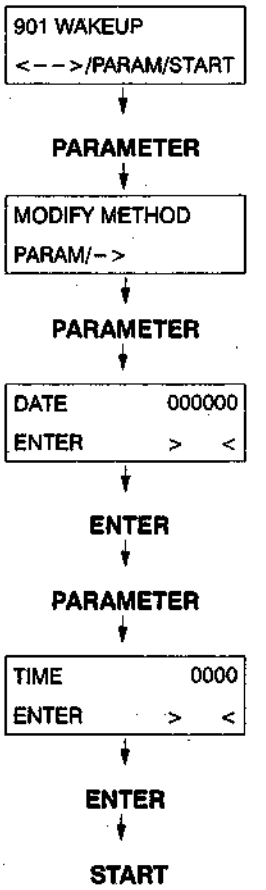
5) Press **[ENTER]** to confirm the entry.

6) Press **[PARAMETER]** again.

7) Type in the 'wakeup' time using the numeric keys; hours, minutes. e.g. 0655.

8) Press **[ENTER]** to confirm the entry.

9) Press **[START]** to activate the wakeup.

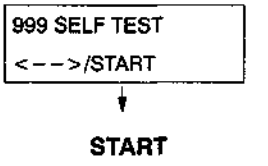


When this method is activated the lamps are switched off and are then switched on again at the preselected WAKEUP *time.*
To exit the WAKEUP *method press* **[STOP]** *and the display returns to standby. Both lamps go on.*

5.13 Self Test

The spectrometer tests the signals from the optics electronically, and prints a report at the end of the test. We recommend you do this test after installing a new lamp.

1) Select the SELF TEST method (999).



2) Press **[START]** to activate the self test.

... or...

Press **[STOP]** to cancel.

If there are any FAIL results in the self test report then repeat the test.

If you have any further enquiries contact your Perkin-Elmer office.

Operating with Accessories 6

Operating with Accessories 6

6.1 General

Accessories are components, or instruments, that are installed or connected in the sample compartment, or otherwise connected to the spectrometer. For some of these accessories parameters have to be taken into account in the methods.

The accessories described below have parameters in the various methods.

6.2 Accessories

Samples can be applied either manually or with the help of a number of accessories.

The following accessories are currently available:

Changers	5Cell Changer
	6Cell Changer
	8Cell Changer
	9Cell Changer
	13Cell Changer
Sippers:	Vacuum Sipper or Peristaltic Sipper
Autosamplers:	AS-90/91

6.3 Requirements for Operation with Accessories

The following preconditions must be fulfilled in order to operate with accessories:

- The accessory board and connector panel for the accessory in question must be installed in the spectrometer.
  The various circuit boards and connector panels are described in the "Installation, Maintenance and System Description" handbook.
- The accessory in use must be properly selected in the appropriate method.

6.4 Using Methods with Accessories

When an accessory board is installed, an additional parameter, ACCESSORY, appears in the method.

- When the ACCESSORY parameter appears, select the appropriate accessory type (*see Section 10.1, page 10-1*).

Note: The method can still be used even if the accessory in question is not connected; the spectrometer simulates operation with the accessory.
However, with cell changers only one sample can be measured.

6.4.1 Spectrometer without Accessory Board

Spectrometers without an accessory board cannot be operated with accessories, but the method parameters for accessories can be selected. The methods can be stored and used on spectrometers with the necessary equipment.

The procedure for setting up a method for an accessory although an accessory board is not installed is as follows:

1) Switch on the spectrometer in Super User mode (*see Appendix 1*).
2) Select the CONFIGURATION branch.
3) Select method 7 ACCESSORY CONFIG.
4) Modify the method by selecting ACCESSORY = YES.
5) Press **[START]** to store the changes and to activate the accessory mode.
6) To deactivate the Super User mode, switch off the spectrometer.
7) Wait about two minutes to allow the lamps to cool down.
8) Switch on the spectrometer.
9) Set up the method as required.

6.5 Running Methods with an Accessory

When using methods with accessories, the spectrometer automatically presents the necessary actions, e.g.:

BACK CORR IN CELL1 PRESS START	Insert blank at location 1 of the cell changer, and then press **[START]**.
SAMPLES IN 9–CELL PRESS START	Insert samples in the cell changer, and then press **[START]**.
BACK CORR SIPPER ACCESSORY START	Press **[START]** on the Sipper (background correction).
SAMPLE 1 SIPPER ACCESSORY START	Press **[START]** on the Sipper (sample measurement).

6.6 Background Correction with Cell Changers

The locations used for sample measurement depend on the tagging and option chosen for the BACK CORR parameter. Not all locations can be used for sample measurement, *see Table 6-1*:

Table 6-1 BACK CORR Tagging

	BACK CORR tagging		
	FIX / CALL	START	BATCH
Procedure	Insert solutions according to display.	When selecting a background correction: – First insert all solutions – Then select YES or NO and press **[START]**.	If no background correction is selected, BACK CORR is only selectable in the appropriate method! Thus, on demand to insert the sample: – First insert all solutions – Then start the first measurement.
Locations			
Blank solution	1	1	1
Sample solution			
BACK CORR=YES	1 ... n	2 ... n	2 ... n
BACK CORR=NO	1 ... n	2 ... n	1 ... n

6.7 CONCENTRATION Methods with Cell Changers

Please note the following when using CONCENTRATION methods:

- When BACK CORR = YES has been selected, background correction is carried out once the method has been started, independent of whether the parameter has been tagged or not.
- Insert the reference solutions in sequence when asked. Always start at location 1.

e.g.

```
REFS IN CELL 1–5
PRESS START
```

6.8 Accessory Parameters

Parameter	Description
General	
ACCESSORY	Select the accessory. MANUAL: operation with standard cell holder, no accessories CELL: for cell changers e.g. 5Cell Changer 6Cell Changer 8Cell Changer 9Cell Changer 13Cell Changer SIPPER: for Vacuum Sipper or Peristaltic Sipper AS–90/91: for autosamplers AS-90 and AS-91 *Note: Sipper operation is not possible when using SUBSTRATE methods. If ENZYME methods are used, neither Sipper nor Autosampler can be used.*
Cell changer (5cell, 6cell, 8cell, 9cell)	
CELL 1-n	The locations at which measurements are to take place. Enter the number of the locations and press **[ENTER]**. If e.g. measurement is to be carried out at locations 2 and 5 only, enter **25**. If BACK CORR = YES has been selected, location 1 is used for background correction, independent of the locations selected as described above. Thus: If BACK CORR = YES has been selected, place the blank at location 1 and the sample solutions from location 2. If BACK CORR = NO has been selected, all locations can be used for sample measurement.

Parameter	Description
Cell changer (13Cell Changer)	
CELL 1-7	The locations at which measurements are to take place.
CELL 8-13	When CELL 1-7 shows, the numbers 1 to 7 represent sample locations 1 to 7. When CELL 8-13 shows, the numbers 1 to 6 represent sample locations 8 to 13. If none of the locations is to be used, enter **0**. Press **[ENTER]** to confirm the location numbers. If e.g. measurement is to be carried out at locations 2 and 5 only, enter **25** and then press **[ENTER]** (CELL 1-7), and enter **0** and then press **[ENTER]** (CELL 8-13). If BACK CORR = YES has been selected, location 1 is used for background correction measurement, independent of the locations selected as described above. Thus: If BACK CORR = YES has been selected, place the blank solution at location 1 and the sample solutions from location 2. If BACK CORR = NO has been selected, all locations can be used for sample measurement. Example 1: CELL 1-7 = 246 Measurement will take place at CELL 8-13 = 0 locations 2, 4 and 6; the rest will not be used. Example 2: CELL 1-7 = 567 Measurement will take place at CELL 8-13 = 123456 locations 5 to 13.
STIRRER	Switches the magnetic stirrer on and off. Option: YES NO Select with arrow key. If the magnetic stirrer has been switched on, place a small magnetic stirring bar in each of the cells. The arrangement is such that whilst measurement is taking place in the one cell the following cell will be stirred.

Parameter	Description
SAMPLING TIME	Sample aspiration time in seconds for the sipper. Range: 0.1 to 99.9 Enter value and confirm by pressing **[ENTER]**.
DELAY TIME	Delay between the end of the aspiration process and the start of the measurement. Range: 0.0 to 99.9 Enter value and confirm by pressing **[ENTER]**.
AUTO PURGE	Switches the autopurge function on and off. Option: YES NO Select with arrow keys.
RETURN *(Peristaltic Sipper only)*	Pump in reverse direction after sample measurement. Option: YES NO Select with arrow keys.
OPTIMIZATION *(Peristaltic Sipper only)*	To optimize the RETURN function. Option: YES NO Select with arrow keys.
REV. TIME *(Peristaltic Sipper only)*	To enter the reversed flow time for the optimization. Range: 0.0 to 99.9 Enter value and confirm by pressing **[ENTER]**.
SIPPERS	Parameters for control of the sipper must be entered via the sipper (*see sippers user documentation*).
AS-90/91 + SIPPER	Parameters and operation with the AS-90/91 are described in the AS-90/91 user documentation.

Care 7

Care 7

W01.02

WARNING

Unauthorized Adjustments and Servicing

Do not attempt to make adjustments, replacements or repairs to this instrument except as described in the accompanying User Documentation.

Only a Perkin-Elmer service representative or similarly trained and authorized person should be permitted to service the instrument.

7.1 Daily Care

- Do not leave samples, particularly those given to fuming or evaporation, in the sample compartment for longer than necessary.
- If any type of sample handling system is installed and portions of it are left in the sample compartment (such as a sipper and flowcell), make certain that the system is cleaned at the end of the working day.
  Generally, such systems should be filled with deionized water when left overnight.
- Immediately clean all spilled materials from the affected area and wipe it dry with lintless paper or cloth.
  If you have to wipe sample compartment windows, make sure you do not introduce scratches. Sample windows are optical components and you should handle them in the same way as high quality cells.

CAUTION

Take care not to spill liquids onto the spectrometer. Expensive damage can result to the optics or electronics if liquids are spilled and run inside the instrument or onto the keyboard.

7.2 Use and Care of Cells

7.2.1 Cell Handling

- Only hold cells by non-optical surfaces, such as the matt finish surfaces.
- Always wipe the optical surfaces of cells dry and free of fingermarks, using a soft lint free cloth or cleaning tissue, just before placing them in the cell holder.
- Protect cells from scratches, and never permit them to rub against one another or against other hard surfaces.
- Avoid abrasive, corrosive or stain-producing cleaning agents, and make sure that the exposed surfaces of cells are optically clean.
- When measuring cold solutions, always bear in mind that condensation can form on the optical surfaces.
- Make sure no bubbles cling to the inner surfaces of the cell, particularly when handling cold solutions.
- For maximum precision and accuracy, calibrate and test with cells of the same type, and always insert cells into the holders with the same orientation.

7.2.2 Pressure Buildup in Cells

- Only fill the cell so full that the liquid meniscus is just above the radiation beam. The remaining air space in the cell is then adequate to compensate for any slight increase in pressure in the cell during routine operation.
- If, for analytical reasons, it is necessary to fill the cell completely, insert the stopper only lightly so that the liquid in the cell has a chance to expand.
- Do not insert a stopper forcefully into a completely filled cell since this is likely to cause the cell to burst.
- When working at higher temperatures, use a drilled stopper (0.4 mm hole) to allow for expansion in the cell.

7.3 Sample Compartment Windows

- Generally, the windows should be installed at all times.
- The windows are optical components and require the same care and handling as cells.
- You can remove the windows to clean them, they are held in place by a magnetic frame.
  Windows are most suitably cleaned by wiping them with a soft lint free cloth moistened with ethanol.

Analytical Notes

8

Analytical Notes 8

8.1 Background Correction

The type of background correction depends on the method type selected.

In methods with a fixed wavelength e.g. TIME DRIVE, WAVELENGTH PROGRAM, CONCENTRATION 1 the displayed measurement value for absorbance is set to 0, for transmission to 100%, at the measurement wavelength (this is called an autozero).
You can use the **[BACK CORR]** key to perform a manual autozero in fixed wavelength methods (*see Section 3.3, page 3-3*).

In methods with measurement over a wavelength range e.g. SCAN, CONCENTRATION 2 a background correction is performed over the selected wavelength range.
A background correction can only be performed in a method.

The ordinate mode of the last used method always appears on the display. To change from absorbance to transmittance or vice versa, select a TIME DRIVE *method, and then select the desired ordinate mode.*

A background correction, or autozero, must be performed:

- at the start of a new method,
- when the wavelength is changed,
- when the wavelength range is extended,
- each time the solvent is changed.

To perform a background correction, or autozero, place cells with a blank solution (or reference solution) in the sample cell holder and reference cell holder.

8.2 Unusual Samples

If a sample is chemically stable and undergoes no physical or chemical change other than to absorb incident radiation, errors in photometric values should not be caused by the sample. Many samples are not this stable, and special consideration must be given to them.

1. Volatile Samples

Some liquid samples are so volatile that their concentration can change while recording is in progress. If this occurs, the resulting data will lack reproducibility.
If you are analyzing volatile samples, use stoppered cells to prevent this problem.

2. Samples not Governed by the Beer-Lambert Law

Quantitative analyses utilizing the absorption of spectral radiation are based on the Beer-Lambert law which states that the absorption is proportional to the concentration of the analyte.

The law can be expressed in the form

$$A = \varepsilon c\, d$$

where

A is absorbance
ε is molar absorption coefficient
c is molar concentration
d is thickness through which the radiation is transmitted

This law is mostly true for dilute solutions, but at higher concentrations a plot of absorbance against concentration will be nonlinear for a number of reasons.

The absorption characteristics of a sample can be changed during sample preparation, depending on the amount of reagent added for color development and so on. For details, refer to reference books covering these subjects.

Temperature has an influence to a greater or lesser degree on the absorption characteristics of a sample. You should check this effect if non-repeatable results are obtained.
If you are measuring temperature-dependent samples, either wait until temperature equilibrium has been attained or use a thermostatted cell or cell holder.

3. Chemically Reactive Samples

If a reaction takes place in the cell between the sample material and the solvent, spectral data based on that sample cannot always be expected to have sufficient reliability or repeatability.
For samples of this type, use a quantitative method that takes advantage of the change in transmittance with time at a fixed wavelength.
For details, refer to reference books covering this specific subject.

4. Photoactive Samples

Some samples are known to be photoactive in that they fluoresce upon absorbing radiation. Since a small portion of the fluorescent radiation will be measured by the detector, a higher apparent transmittance will often result.
Samples are also known that undergo photochemical reactions as they absorb radiation. With such samples, which are mostly biochemical, lack of reproducibility will characterize the resultant data.

5. Other Sample Properties

Samples that are polarizing in nature, or have a double index of refraction, are often difficult to measure accurately. The emerging monochromatic radiation is slightly polarized due to having been refracted.

Thin-film samples also pose a problem since optical interferences may develop, causing a regular interference pattern to be superimposed on the spectral curve.

8.3 Solvent Properties

The solvent should meet the following requirements:

- It should dissolve the sample without reacting with it.
- The radiation absorption in the scanning region should be low. High absorption by the blank reduces the reference energy, thus increasing noise.
- Evaporation should be fairly low at ambient temperature.

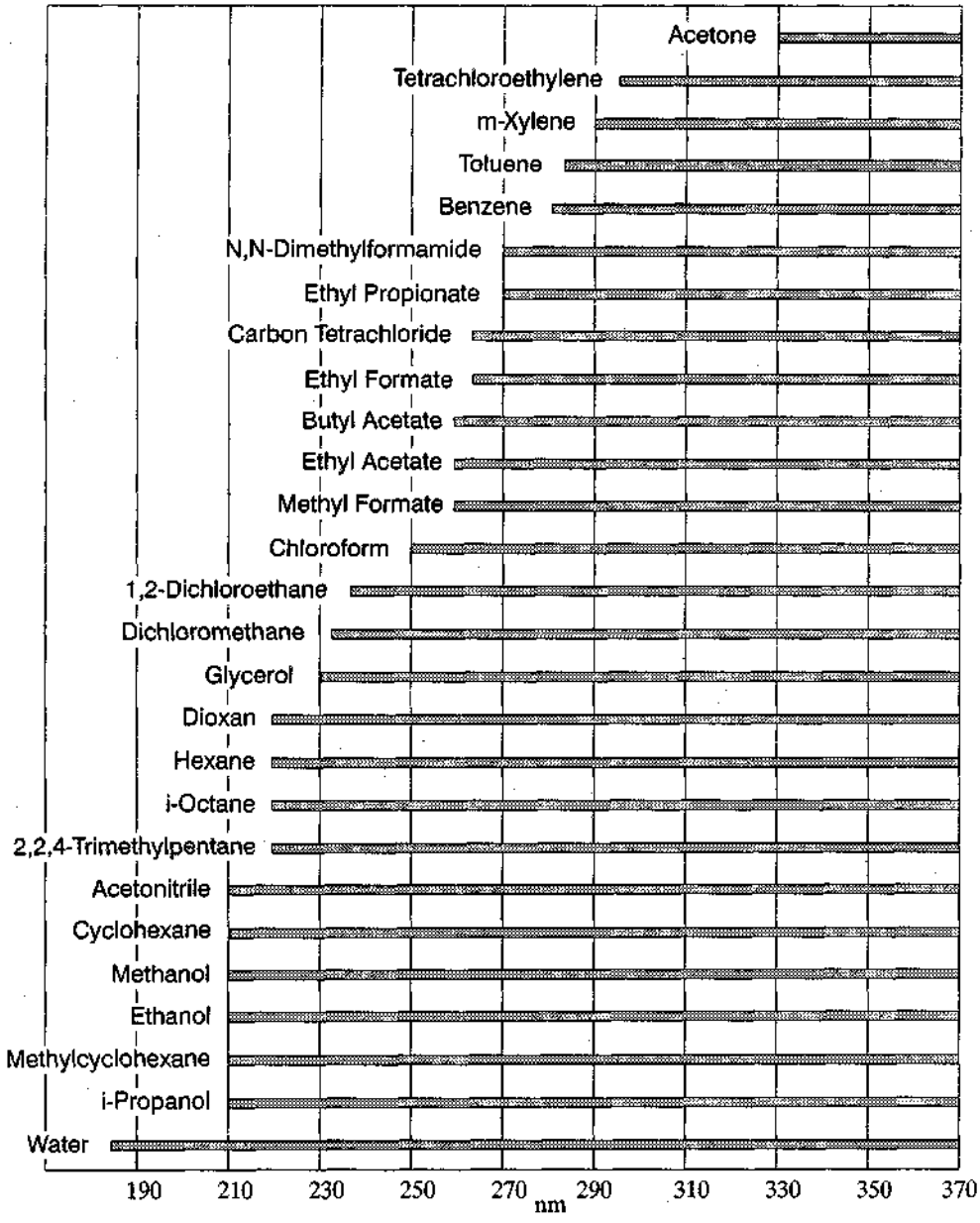
In general, aromatic compounds exhibit high absorption in the UV region and hence are not suitable as solvents for measurements in this region.

Water is virtually the only useful solvent below 195 nm, but it must be freed from oxygen to attain best transmission.

Whenever you are going to use a solvent with unknown absorption characteristics, scan its spectrum first to determine whether it is suitable.

The lower wavelength limits of a number of commonly used solvents are presented in the following table.
The lower limit has been defined as that wavelength at which 10 mm of pure solvent has a transmission of 10 %.

Table 8-1 Lower Wavelength Limits of Solvents



Error Messages

9

Error Messages 9

If an error occurs during the operation of the spectrometer, an error message is shown on the display or is printed out (if a printer is connected).

9.1 Error Messages Shown on the Display

Errors remain displayed until they are deleted. To delete, press **[PARAMETER]**.

Error	Meaning
RANGE ERROR: xxxx.x – xxxx.x	The value entered is outside the displayed range. Press **[PARAMETER]** and enter a value within the range shown.
PARAMETER XX: DOES NOT EXIST	The parameter XX does is not exist. Press **[PARAMETER]** to continue.
PARAMETER XX: NOT USED	The parameter XX is not used, or is not active in this method. Press **[PARAMETER]** to continue.
HINT: INT.GREATER TOT.TIM	The **Interval** time is **greater** than the **total time**. This message appears with kinetic measurements. Select a shorter interval time.
HINT: SELECT INTERVAL TIME	Appears with enzyme methods with cell changers, if the interval time does not correlate with the measurement time. Select the INTERVAL time so that the TOTAL TIME can be divided by the INTERVAL time evenly.

Error	Meaning
PROBLEM: MARK NOT SET	COPY FROM MARK was selected without first initiating MARK FOR COPY. First use MARK FOR COPY and then select COPY FROM MARK.
PROBLEM: METHOD NOT FOUND	A method has been tagged MARK FOR COPY although the method in question is no longer available.
PROBLEM: ACCESSORY NOT INITIALIZED	This error is shown when the instrument gets no response from an attached accessory during startup. Check that the accessory is: – correctly connected – switched on – functioning normally
PROBLEM: METHOD NO. LIMITS 1-999	The method number entered is outside the displayed range. Enter a number within the range 1 to 999.
ERROR: LAST METHOD	An attempt was made to delete all methods. Retain at least one method in the memory otherwise the spectrometer cannot work.
PROBLEM: METHOD PROTECTED	An attempt was made to select a fully protected method. The protection has to be modified if the method is to be used.
DON'T PROTECT ALL METHODS	An attempt was made to ALL protect all the methods. Retain at least one method otherwise the spectrometer cannot work.
DON'T PROTECT ALL BRANCHES	An attempt was made to ALL protect all branches. Retain at least one branch otherwise the spectrometer cannot work.
PROBLEM: BRANCH WRITE PROTECT	An attempt was made to modify methods in a write-protected branch. To modify the method, alter the branch protection.

Error	Meaning
PROBLEM: DIRECTORY FULL	An attempt was made to store more than 200 methods. To create space for the new method, delete a method that is no longer required.
PROBLEM: MEMORY FULL	The available memory is insufficient to cope with the new method. To create space for the new method, delete a method that is no longer required.
ERROR: NO ENERGY	This error is shown when not enough energy is detected. Possible causes: – Beam is blocked in the sample compartment – Loose lamp connection – Lamp burnt out – Lamp(s) switched off – Defective detector
ERROR: NO ENERGY, UV LAMP	This error is shown when not enough energy is received from the UV lamp. Possible causes: – Beam is blocked in the sample compartment – UV lamp loose connection – UV lamp burnt out – UV lamp switched off – Defective detector
PROBLEM: NO ENERGY, VIS LAMP	This error is shown when not enough energy is received from the Vis lamp. Possible causes: – Beam is blocked in the sample compartment – Vis lamp loose connection – Vis lamp burnt out – Vis lamp switched off – Defective detector

Error	Meaning
PROBLEM: SYSTEM ERROR	This error is shown when the instrument operating software "crashes". A full reset is then automatically carried out. After the instrument is reset one of the following messages is shown on the second line of the display: BATTERY LOW TIMER FAIL RS232-IRQ FAIL TIMER-IRQ FAIL Make a note of this message. Press **[PARAMETER]** to continue. If you cannot continue call your Perkin-Elmer office and inform them of the error message. *Note: After a full reset all methods are erased.*
SPECTROMETER FULL RESET DONE	This error is shown after changing the instrument software, or after a full reset, or when the spectrometer data is defect. Make a note of the steps you made leading up to this message. Press **[PARAMETER]** to continue. If you cannot continue call your Perkin-Elmer office and inform them of the error message and the steps you made leading up to the error. *Note: After a full reset all methods are erased.*
DIALOG, FULL RESET DONE	This error is shown after changing the instrument software, or after a full reset, or when the method memory is defect. Make a note of the steps you made leading up to this message. Press **[PARAMETER]** to continue. If you cannot continue call your Perkin-Elmer office and inform them of the error and the steps you made leading up to the error. *Note: After a full reset all methods are erased.*

Error	Meaning
SPECTROMETER + DIALOG FULL RESET DONE	This error is shown after changing the instrument software, or after a full reset. Make a note of the steps you made leading up to this message. Press **[PARAMETER]** to continue. If you cannot continue call your Perkin-Elmer office and inform them of the error and the steps you made leading up to the error. *Note: After a full reset all methods are erased.*
BUS ERROR + a *message*	This error, plus a message, is shown when the instrument has an address error. Make a note of this message and the steps you made leading up to the error. Press **[PARAMETER]** to continue. If you cannot continue call your Perkin-Elmer office and inform them of the error, the error message and the steps you made leading up to the error. *Note: After a full reset all methods are erased.*

9.2 Error Reports on the Printer

Error	Meaning
Baseline correction data do not fit. Start baseline correction.	The background correction last carried out did not correlate to the method used. Carry out a new background correction with the proper method.
Cannot approximate calibration curve. Check references or change curve fit algorithm.	The calibration data deviate strongly from the curve form selected or not enough points were measured for the curve form selected. Check the references, select another curve form or carry out more measurements.
Cannot calculate delta absorbance. Because too few points read.	A kinetic method was interrupted with **[STOP]**. Too few points available to calculate delta absorbance.
Cannot calculate slope. Because too few points read.	A kinetic method was interrupted with **[STOP]**. Too few points available to calculate the slope.
End time out of limits. Change end time.	In recalculation, an out of limits value for END TIME was entered. Enter a lower END TIME value and repeat the procedure.
Lag or total time is not divisible by interval time (0.0).	In recalculation, the LAG TIME or TOTAL TIME entered was not divisible by the INTERVAL time. Enter a value for LAG TIME or TOTAL TIME that is fully divisible by the INTERVAL time.
Lag or total time out of limits. Change lag and/or total time.	In recalculation, a greater value for LAG TIME or TOTAL TIME was entered than the actual measuring time. Enter a lower value for LAG TIME or TOTAL TIME.
Lag time greater than total time. Change lag time?	In recalculation, a value for LAG TIME was entered that was greater than the measuring time. Enter a LAG TIME less than the TOTAL TIME.

Error	Meaning
More than one peak within wavelength limits. Change threshold or measurement wavelengths.	More than one peak identified within the selected measuring range. Change WAV. MIN and WAV. MAX or THRESHOLD so that only one peak is detected.
No peak detected. Change threshold.	The THRESHOLD is too high to detect peaks. Select a lower value for THRESHOLD.
No points stored. Start measurement.	The method was interrupted with **[STOP]** before data could be stored, e.g. during the equilibrium time of a substrate method. Restart the method.
Two solutions for 1 ABS value. Change curve fit algorithm.	In a non-linear calibration curve two reference solutions exhibit the same absorbance value. Check the references, or change the concentration range, or change the curve fit algorithm.
Value not within valid limits. Check references or change curve fit algorithm.	The measured sample concentration is outside the calibration range. Measure additional calibration solutions within the concentration range or change the curve fit algorithm.
Wavelength data do not fit. Start background correction.	Appears with TIME DRIVE methods if the wavelength has been modified since the last background correction. Perform a new background correction using the proper method.

Parameter Numbers and Descriptions

10

Parameter Numbers and Descriptions

10

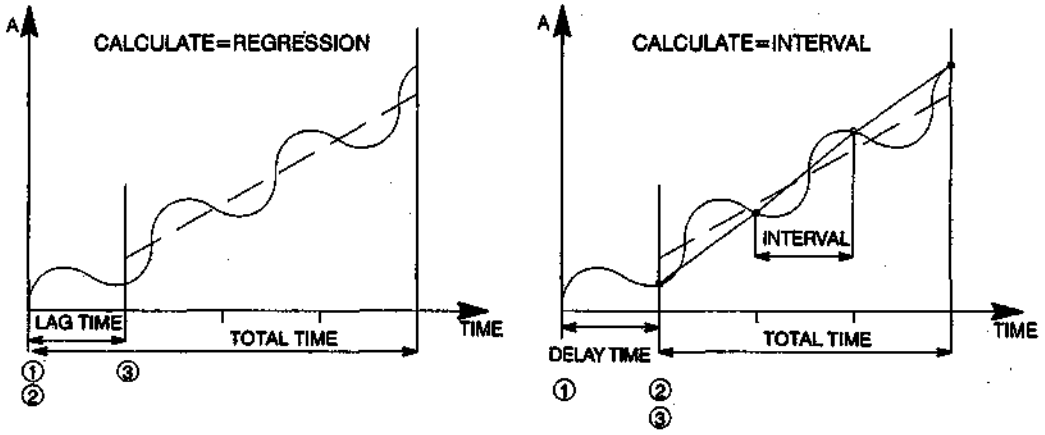
10.1 Parameter Numbers and Descriptions

Parameter	No.	Description
# OF REFS	2	Number of reference solutions used for the calibration. Range: 1 to 20
# WAVELENGTHS	2	Number of wavelengths at which measurements are made. Range: 0 to 20 If ordinate modes RAT and DIF are used, the number must be divisible by 2. If ordinate mode COR is used, the number must be divisible by 3. *See also parameter Ordinate Mode.*
ACCESSORY	38	Lets the spectrometer recognize the **accessory** type connected. **MANUAL** is for operations without using an accessory, or for manual cell changers. **CELL** is for a cell changer. **SIPPER** is for a sipper. **AS-90/91** is for the AS-90/91 autosampler. *Note: This parameter only shows when an accessory board is installed in the spectrometer.* *Sipper operation is not possible when using SUBSTRATE methods.* *If ENZYME methods are used, neither sipper nor autosampler can be used.*
AUTO METHOD	35	Prints method parameters prior to each method start. Option: YES NO
AUTO PURGE	48	Switches the autopurge function on and off. Option: YES NO

Parameter	No.	Description
BACK CORR	16	Background correction. Option: YES NO Without tagging, background correction should be carried out at the start of the method. If tagging has been carried out, background correction is offered: – At the start of the method (CALL tagging) – Prior to each sample batch (BATCH tagging) – Prior to each sample (START tagging) **Time Drive, Wavelength Prog.** Background correction is carried out at every wavelength, i.e. the basic signal is set to zero. **Scan.** Background correction is carried out within the range WAV. MAX and WAV. MIN, i.e. the baseline is set to zero. **Concentration 1, Concentration 2.** If BACK CORR = YES has been selected, background correction must be carried out prior to measurement of the first reference solution. Background correction should be carried out at wavelengths WAVELENGTH 1-3, i.e. the baseline signal is set to zero at these points. **Substrate, Enzyme.** Background correction is carried out at the wavelength selected.

Parameter	No.	Description
BLANK	7	Blank value. **Enzyme.** Blank value (units as selected for ENZ UNIT). Range: 0.00001 to 9999.9 The enzyme activity is calculated as follows: $a_{Enz} = a_{Total} - a_{Blank}$ **Substrate.** Blank value (units as selected for CONC UNIT). Range: −9999.9 to 9999.9 The substrate concentration is calculated as follows: $c_{Sub} = c_{Total} - c_{Blank}$
CALC. WAV 1	5	Wavelength in nm (used in CONCENTRATION 2 methods for calculations using the 2nd order derivative only). Range: 190.0 to 1100.0, in steps of 0.1 The values must lie within the WAV. MAX and WAV. MIN values set. Enter the value at peak maximum for CALC. WAV 1, *see page 5-20*. In MODE = DERIV 2 FIX, the height of the derivative curve is measured at these wavelengths. In MODE = DERIV 2 PEAK, a derivative maximum or minimum is located around these wavelengths and the nearest one evaluated (*see also parameter MODE*).
CALC. WAV 2	5	Wavelength in nm (used in CONCENTRATION 2 methods for calculations using the 2nd order derivative only). Range: 190.0 to 1100.0, in steps of 0.1 The values must lie within the WAV. MAX and WAV. MIN values set. Enter the value at peak minimum for CALC. WAV 2, *see page 5-20*. In MODE = DERIV 2 FIX, the height of the derivative curve is measured at these wavelengths. In MODE = DERIV 2 PEAK, a derivative maximum or minimum is located around these wavelengths and the nearest one evaluated (*see also parameter MODE*).

Parameter	No.	Description
CALCULATE	9	Slope calculation mode: REGRESSION: The slope is calculated using all data points by means of linear regression. INTERVAL: The slope is calculated for each interval. The mean of all the slopes is then used for the calculation of enzyme activity. *Note: When using CALCULATE = INTERVAL, please bear in mind:* *Instead of the parameter LAG TIME, parameter DELAY TIME (equilibration time) appears.* *Measurement begins only when the DELAY TIME has elapsed.* *The TOTAL TIME must be a whole multiple of the INTERVAL time.*

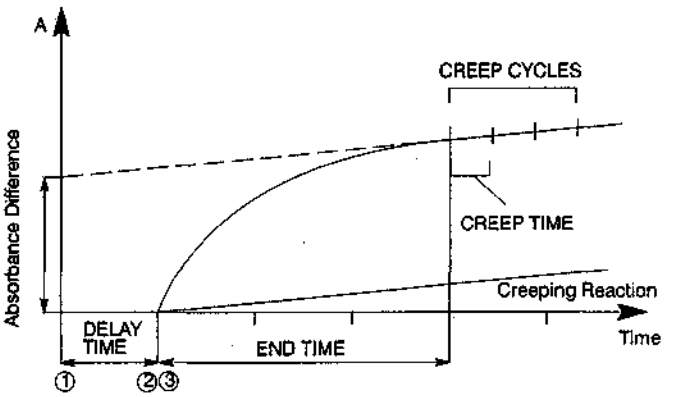


① Start of the Method ② Start of Measurement ③ Start of Calculation

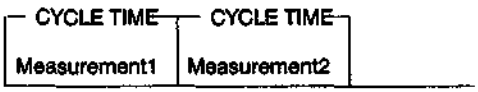
Parameter	No.	Description
CELL 5	**39**	To enter the location of the cell, or cells, to be measured in the 5cell changer.
CELL 6	**40**	To enter the location of the cell, or cells, to be measured in the 6cell changer.
CELL 8	**43**	To enter the location of the cell, or cells, to be measured in the 8cell changer.
CELL 9	**44**	To enter the location of the cell, or cells, to be measured in the 9cell changer.
CELL 1-7	**41**	To enter the location (1 to 7) of the cell, or cells, to be measured in the 13cell changer. Example: Cell 1-7 = 2 4 6 — Measurement will take place at locations 2, 4 and 6; the rest will not be used. Cell 8-13 = 0
CELL 8-13	**42**	To enter the location (8 to 13) of the cell, or cells, to be measured in the 13cell changer. Example: Cell 1-7 = 5 6 7 — Measurement will take place at locations 5 to 13. Cell 8-13 = 1 2 3 4 5 6
CHNG CONST	**65**	To change the molecular mass and molar absorption coefficient values of oligonucleotide bases. Option: YES NO *Shows only when T_M (theoretical melting point) is YES.* *Refer to the Biochemical Application manual.*

Parameter	No.	Description
CONC FACTOR	11	Concentration factor. Range: 0-00001 to 9999.9 *Note: The concentration factor is calculated as follows:* $$Concentration\ Factor = \frac{V \times M}{d \times v \times 1000}$$ *where:* *V is the volume of the total solution in the cell in mL* *M is the molar mass of the substrate in g/mol* *d is the pathlength in cm* *v is the volume of sample in mL* *1000 is the conversion factor for volume units in liters* *Depending on the procedure used, the molar absorption coefficient may need to be taken into account.*
CONC UNIT	6	**Concentration Unit**, defines the concentration unit used for the analysis. **C** is any concentration unit. **g/L** is gram per liter. **mg/L** is milligram per liter. **mg/mL** is milligram per milliliter. **mg/dL** is milligram per deciliter. **µg** is microgram. **µg/mL** is microgram per milliliter. **mol** is mole. **mmol** is millimole. **nmol** is nanomole. **pmol** is picomole. **ppm** is parts per million (mg/kg). **ppb** is parts per billion (µg/kg). **%** is percent. **A1%** is the absorbance of a solution containing 1 g substance in 100 mL of solution, in a cell of 1 cm pathlength. **APHA** is color number. *The units may appear in all capitals on the display.*

Parameter	No.	Description
CREEP CYCLE	**21**	Number of cycles after the reaction end time. Range: 0 (no further measurements after end point) 2 to 99 This parameter is used to compensate for creeping reactions: The spectrometer calculates the slope for each interval. If it remains constant, the substrate reaction is complete; the spectrometer will then determine the difference in absorbance for the substrate reaction. If the slope does not remain constant, two further measuring intervals are added.
CREEP TIME	**24**	Duration of the measuring interval (units as selected for TIME UNIT). Appears only if CREEP CYCLES is 2 or more. Range: 0.1 to 999.9



① Start of the Method ② Enzyme Added ③ Start of Measurement

Parameter	No.	Description
CUR FIT	10	Different types of calibration **Curve Fit** can be calculated by the spectrometer software. LINEAR — used when the measured values vary linearly with the concentration, the curve passes through the origin. LINEAR INTERC — used when the measured values vary linearly with the concentration, the curve has an intercept on the measured value axis to compensate for background interferences. QUADRATIC — used when the measured values do not vary linearly with the concentration, the curve passes through the origin. QUAD INTERC — used when the measured values do not vary linearly with the concentration, the curve has an intercept on the measured value axis to compensate for background interferences.
CYCLE TIME	22	The time in minutes between the start of one sample measurement to the start of the next sample measurement. Range: 0.002 to 999.99  Duration of the analysis = CYCLES × CYCLE TIME *Note: When using accessories, set the CYCLE TIME longer than required for scanning the spectrum. This avoids time problems.*
CYCLES	21	The number of times one sample is scanned or measured. Range: 1 to 99 *Note: If CYCLES is set to 0, the sample is scanned or measured continuously until you stop the method.*

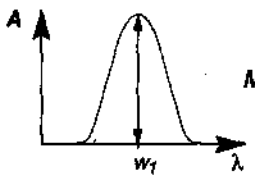
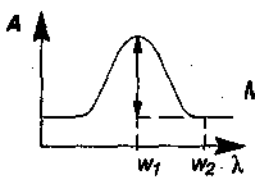
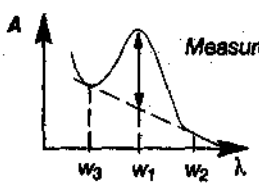
Parameter	No.	Description
DELAY TIME	**23**	Equilibration time (Only in the case of use with the cell holder or in manual operation with CALCULATE = INTERVAL). This is the time from the start of the method to the start of measurement (units as selected for TIME UNIT). Range: 0.0 to 999.9 *Note: Measurement begins after equilibration time has elapsed.* **Substrate.** Once the equilibration time has elapsed, add the enzyme solution to the cell and mix.
	47	**Operating with a Sipper.** Delay between the end of the aspiration process and the start of the measurement. Range: 0.1 to 99.9
DIL FACTOR	**10**	Dilution factor. Range: 0.00001 to 9999.9
DIVISOR	**12**	Divisor. Range: 0.00001 to 9999.9 *For example the molar absorption coefficient value can be entered as divisor. Values can be obtained from the literature.* *Note: If the absorption coefficient is already included in the ENZ FACTOR (enzyme factor), enter DIVISOR = 1.* **Enzyme.** Enzyme activity is automatically calculated as follows: a_{Enz} = enzyme factor x dilution factor x 1/divisor x dA/dt **Substrate.** Substrate concentration c_{Sub} is calculated as follows: c_{Sub} = concentration factor x dilution factor x 1/divisor x ΔA with ΔA = absorbance difference.

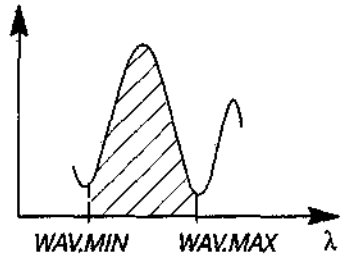
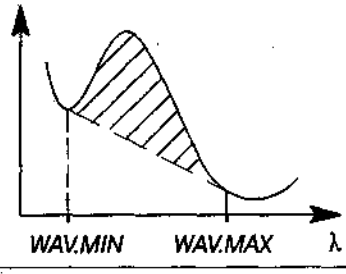
Parameter	No.	Description
DIVISOR (continued)	12	**Concentration 1, Concentration 2.** Divisor. The measured value is multiplied by the factor or divided by the divisor and the resultant value displayed. Thus, dilution procedures or differing masses can be taken into account. If a dedicated correction factor is to be used for each sample, select FACTOR or DIVISOR as a START (tag) parameter (*see page 4-7*). The factor or divisor can be entered immediately prior to each analysis. Example: A particular component of a powder is to be determined and displayed in mg/g. The calibration curve is compiled using pure substance in solution made up in mg/L. In order that the results can be displayed independently of the actual mass of powder used, the mass of the powder should be entered as the divisor. If the powder were dissolved in 0.25 L instead of in 1 L, an additional dilution factor of 0.25 should be entered. The results are then automatically calculated as follows: (0.25/mass of powder in g) x concentration in mg/L = concentration in mg/g
END TIME	22	Time from the start of the reaction (i.e. addition of enzyme) to the end of the reaction (units as selected for TIME UNIT). Range: 0.1 to 999.9
ENZ FACTOR	11	Enzyme factor. Range: 0.00001 to 9999.9 Calculate the enzyme factor as follows: Enzyme factor = V/dv where: V is the volume of the total solution in the cell in mL d is the pathlength in cm v is the volume of sample in mL ***Note:*** *Depending on the procedure used, the molar absorption coefficient may need to be taken into account.*

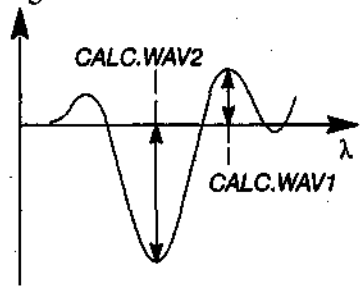
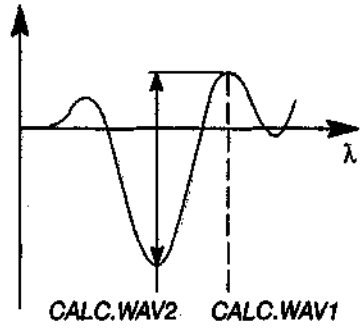
Parameter	No.	Description
ENZ UNIT	6	**Enzyme Unit.** **U/L** is units per liter. **U/mL** is units per milliliter. **mU/L** is milliunits per liter. **U** is units. **mU** is milliunits. **mg/mL** is milligrams per milliliter. **--** is any unit. *Enzyme Unit U is the amount of enzyme which catalyzes the conversion of 1 µmol (or micro-equivalent) of substrate per minute.* *The unit may appear in capitals on the display.*
FACTOR	11	Factor. Range: 0.000001 to 9999 The measured value is multiplied by the factor and the result displayed. Thus, concentration can be read off directly or a dilution taken into account. If only the true measured value is to be shown, then choose FACTOR = 1. If concentration units are to be read off directly, calculate the factor according to the Beer-Lambert law: $A = \varepsilon c d$ where A is the absorbance ε is the molar absorption coefficient c is the concentration of the sample d is the pathlength of the cell Thus, the concentration $c = A/(\varepsilon d)$ and the factor $f = 1/(\varepsilon d)$

Parameter	No.	Description
FACTOR n	**11**	Factor (for each wavelength n). Range: -9999.9 to 9999.9 The measured value is multiplied by the factor and the result displayed. Thus, concentration can be read off directly or a dilution taken into account. If only the true measured value is to be shown, then choose FACTOR $n = 1$. If concentration units are to be read off directly, calculate the factor according to the Beer-Lambert law: $A = \varepsilon c d$ where A is the absorbance ε is the molar absorption coefficient c is the concentration of the sample d is the pathlength of the cell Thus, the concentration $c = A/(\varepsilon d)$ and the factor $f = 1/(\varepsilon d)$
FIRST SAMPLE #	**19**	Number of the first sample in the batch. All subsequent samples are automatically numbered consecutively.
GRAPHICS PLOT	**25**	Graphics printout. Option: YES NO
GRID	**29**	Graphics printout with grid. (valid only when GRAPHICS PLOT = YES). Option: YES NO
INTERVAL	**21**	**Interval** time (units as selected for TIME UNIT). Range: 0.1 to 999.9 When using CALCULATE = REGRESSION, the change in absorbance dA/dt is printed out. When using CALCULATE = INTERVAL, the slope is calculated for each interval. The mean of all slopes is then used for the calculation of the enzyme activity (*see also parameter* CALCULATE).

Parameter	No.	Description
INTERVAL (continued)	21	***Operating with the Cell Changer:*** During the interval time the spectrometer measures once at each location. To do this you need to know the minimum measuring time t_{min} : $t_{min} = N \times (3 \times \text{RESPONSE} + x + 0.1)$ seconds where: N = the number of cells 3 × RESPONSE = measuring time per cell x = relocation time from cell to cell The interval time should always be greater than the required minimum time.
LAG TIME	24	Lag time. This is the time from the start of the method to the start of calculation (units as selected for TIME UNIT). After this time a constant reaction rate should have been reached. Range: 0.0 to 999.9 *Measurement begins with the start of the method. However, enzyme activity is only calculated from the end of the lag time.* *(Only when using POSTRUN KIN = YES, or manual operation together with CALCULATE = REGRESSION).*
LAMP	15	Switched on lamps. **UV:** 190 nm to 326 nm **VIS:** 326 nm to 1100 nm **UV/VIS:** 190 nm to 1100 nm *Note: In order to preserve the UV lamp:* *- Switch off the lamp only at the end of the working day.* *- Allow the lamp to cool off for at least 2 minutes before switching on again.*
LINE TYPE	31	Type of line used for the graphics printout (valid only when GRAPHICS PLOT = YES). DASH1: —————— DASH2: — — — — — — DASH3: - - - - - - - - - DASH4: —— · ——— · —— AUTO: For each curve, a different type of line is used in the sequence DASH1, DASH2, DASH3, DASH4, DASH1 etc.

Parameter	No.	Description
MODE	1	**Concentration 1.** Sets the **mode** in which the measurements are made. **ABS** is absorbance **DELTA ABS** is **delta** absorbance **3WL Analysis** is 3 wavelength **analysis** **ABS**: Measuring absorbance at one wavelength:  *Measured value* = A_{w1} **DELTA ABS**: Correction for an offset baseline by measuring at two wavelengths:  *Measured value* = $A_{w1} - A_{w2}$ **3 WL ANLYS**: Correction for a sloping baseline by measuring at three wavelengths:  *Measured value* = $A_{w1} - \frac{(A_{w2} - A_{w3})(w_1 - w_3)}{w_2 - w_3} - A_{w3}$

Parameter	No.	Description
MODE (continued)	1	**Concentration 2.** Sets the **mode** in which the measurements are made. There are four possibilities: **TOTAL AREA** **PEAK AREA** **DERIV 2 FIX** **DERIV 2 PEAK** **TOTAL AREA**: the total peak area between wavelength maximum and wavelength minimum is calculated.  **PEAK AREA**: the peak area between wavelength maximum and wavelength minimum is calculated with a correction for the baseline drift. 

Parameter	No.	Description
MODE (continued)	1	**DERIV 2 FIX**: Height of the 2nd order derivative curve at wavelengths CALC.WAV2 and CALC.WAV1 is measured.  **DERIV 2 PEAK**: Height of the 2nd order derivative peak. The software locates the extreme values around wavelengths CALC.WAV2 and CALC.WAV 1 and calculates the difference in height between these two points. Only values that have exceeded the set THRESHOLD are taken into account. This function can avoid errors that may occur through displacement of the spectrum. 
MMASS dA	66	Sets the relative molecular mass of the Adenine base. Range: 0 to 99999 *Used with the Oligoquant methods.*
MMASS dC	67	Sets the relative molecular mass of the Cytosine base. Range: 0 to 99999 *Used with the Oligoquant methods.*

Parameter	No.	Description
MMASS dG	**68**	Sets the relative molecular mass of the **G**uanine base. Range: 0 to 99999 *Used with the Oligoquant methods.*
MMASS dT	**69**	Sets the relative molecular mass of the **T**hymine base. Range: 0 to 99999 *Used with the Oligoquant methods.*
MMASS dN	**70**	Sets the relative molecular mass of the base. **N** is used to represent any base. Range: 0 to 99999 *Used with the Oligoquant methods.*
NUMBER OF dA	**60**	Sets the number of **A**denine bases. Range: 0 to 999 *Used with the Oligoquant methods.*
NUMBER OF dC	**61**	Sets the number of **C**ytosine bases. Range: 0 to 999 *Used with the Oligoquant methods.*
NUMBER OF dG	**62**	Sets the number of **G**uanine bases. Range: 0 to 999 *Used with the Oligoquant methods.*
NUMBER OF dT	**63**	Sets the number of **T**hymine bases. Range: 0 to 999 *Used with the Oligoquant methods.*
NUMBER OF N	**64**	Sets the number of bases. **N** is used to represent any base. Range: 0 to 999 *Used with the Oligoquant methods.*

Parameter	No.	Description
OLIGO	**78**	To enter the concentration of primer (DNA) in the solution. This is not necessarily the concentration in the cell. Used for the melting point calculation. *Shows only when T_M (theoretical melting point) is YES.* *Refer to the Biochemical Application manual.*
OPER. ID	**36**	User identification up to a maximum 8 characters. User identification appears on each printout.
ORD.MAX	**26**	Maximum ordinate scale range for graphics printout (valid only if GRAPHICS PLOT = YES). Range: −9999.9 to 9999.9 If the analytical value obtained is outside the set values, the latter should be changed. ***Note:*** *Derivative spectra can also have negative values.*
ORD.MIN	**27**	Minimum ordinate scale range for graphics printout (valid only if GRAPHICS PLOT = YES). Range: −9999.9 to 9999.9 If the analytical value obtained is outside the set values, the latter should be changed. ***Note:*** *Derivative spectra can also have negative values.*

Parameter	No.	Description
ORDINATE MODE	1	Ordinate Mode. **%T:** transmittance in percent **ABS:** Absorbance **D1 to D4:** 1st to 4th derivatives of the spectrum (derivative spectra) **RAT:** Absorbance ratio **DIF:** Absorbance difference **COR:** Corrected Absorbance ratio **CONC:** Concentration (*see also* FACTOR) Derivative modes D1 to D4 can be used to resolve overlapping peaks, to reduce interference and to enhance the fine structure of a particular peak. This facilitates the qualitative evaluation of spectra with overlapping peaks and the quantitative evaluation of spectra with undesired background absorption. The derived values obtained are multiplied by 10 for every degree of derivation in order to produce graphics that are easier to interpret. Resolution and noise increase with the degree of derivation. In general, the 2nd derivative is more helpful in this respect than the 1st: the resolution is better and the characteristic maximum of the signal is easy to recognize as a derivative minimum. Should the 1st or 2nd derivatives prove insufficient, the 3rd or 4th derivatives can be used, providing the noise level remains within acceptable limits. The parameters SPEED and SMOOTH influence the quality of derived spectra. In choosing parameter values, take the following into account: - SPEED: Guideline value = peak width in nm x 10. High scan speeds decrease resolution; low scan speeds increase noise. - SMOOTH: In the case of derivation spectra, smoothing exerts a greater influence than in absorbance measurement. Smoothing should thus be kept to a minimum. Guideline value < peak width in nm x 0.5. Derivation is not possible with a degree of smoothing of 0, independent of the ordinate mode selected.

Parameter	No.	Description
ORDINATE MODE (continued)	1	Ratio, difference and corrected ratio (RAT, DIF, COR) modes are determined according to the equations below: $(RAT = \frac{A_1}{A_2}, \frac{A_3}{A_4}, ...)$ $(DIF = A_1 - A_2, A_3 - A_4, ...)$ $(COR = \frac{A_1 - A_3}{A_2 - A_3}, \frac{A_4 - A_6}{A_5 - A_6}, ...)$ *The subscript 1 stands for the first wavelength, 2 for the second etc...*
OVERLAY	**30**	Prints spectra from the same batch onto the same graphics printout (valid only when GRAPHICS PLOT = YES). Option: YES NO *Overlaying graphics printouts, functions only if the printer used has an automatic paper reverse function!* *If* CYCLES = 1, *all the results of a particular batch are printed out sequentially. This enables spectra to be more easily compared than if they are printed out separately.*
PLOT REFS	**25**	Prints out the calibration curve. Option: YES NO
POSTRUN KIN	**34**	Allows you to recalculate the results. Option: YES NO **Enzyme.** When POSTRUN KIN = YES is used, the results for a changed TOTAL TIME and LAG TIME can be recalculated. When using a cell changer, the values for TOTAL TIME and LAG TIME must be a whole multiple of the set INTERVAL time. **Substrate.** When POSTRUN KIN = YES is used, the results for a changed reaction END TIME can be recalculated. When using a cell changer no recalculation is possible.
PRINT DATA	**32**	**Prints** out a table containing analytical **data** when activated. Option: YES NO

Parameter	No.	Description
PRINT REFS	**26**	**Prints** out data from the **ref**erences when activated. Option: YES NO
REFS	**9**	**Reference** solutions. Choice as to whether a calibration curve should be established or not at the start of a method. Option: OLD NEW Old means you wish to use the "OLD" stored calibration curve. New means you wish to make a "NEW" calibration curve. *If a calibration curve is to be used again, or if the values of the curve are to be entered directly, select REFERENCES = OLD (see also parameter VALUE).* *It is often useful to tag REFERENCES e.g. as CALL parameters: select REFERENCES = NEW when selecting the first method and generate a calibration curve.* *At the next call-up, select REFERENCES = OLD. The available calibration curve is used and measurement can start immediately.*
REF n	**7**	Concentration of **ref**erence solution **n**.
RESPONSE	**14**	**Response** time. Time constant in seconds (s). Option: 0.1; 0.2; 0.5; 1; 2; 5; 10 A large value for time constant gives good signal/noise ratios, but can cause undesirable smoothing of the curve, especially with rapidly altering signals. Thus the time constant should be kept as low as possible, but maintaining an acceptable signal/noise ratio. *Note: In analyses involving creeping reactions, the time constant set must be lower than the CREEP TIME set.* *Note: The time constant set must be lower than the INTERVAL in order to calculate dA/dt (see parameter INTERVAL).*
[SALT]	**77**	To enter the **salt** concentration (monovalent cation concentration) of the oligonucleotide sample. Used for the melting point calculation. Unit: mmol/L *Shows only when T_M (theoretical melting point) is YES.* *Refer to the Biochemical Application manual.*

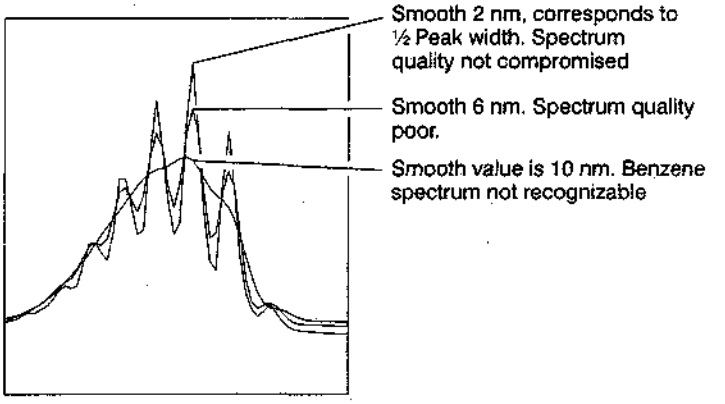
Parameter	No.	Description
SAMPLING TIME		Aspiration time in seconds for sipper. Range: 0.0 to 99.9 *Used with the sipper accessory.*
SAMPLE ID	**37**	Sample identification up to a maximum of 8 characters. Sample identification appears on every printout.
SAMPLES/BATCH	**18**	Number of samples per batch (does not appear when using the cell changer). If batch procedure is not used, enter 0
SCALE	**28**	Abscissa scale in nm/cm or mm/min for graphics printout. (valid only when GRAPHICS PLOT = YES). Options: 0.5; 1; 2; 5; 10; 20; 50; 100 nm/cm 1; 2; 5; 10; 20; 50; 100 mm/min
SEQ.n	**61**	Sequence of the oligonucleotide bases. The sequence must be entered in order from base 5′ to 3′. Use the numeric keys according to the table below to enter the base sequence. Option: **Base** **Key** **A** is Adenine 7 **C** is Cytosine 4 **G** is Guanine 1 **T** is Thymine 0 **N** is any base 8
SEQUENCE LENGTH	**60**	**Length** of the oligonucleotide base **sequence**. Range: 2 to 50
SLIT	**17**	Required slit width in nm. Options: 0.5, 1, 2, 4 ***Note:** Only shows with Lambda 14 and Lambda 14P spectrometers.*

Parameter	No.	Description
SMOOTH	**14**	Smoothing according to Savitzky-Golay in nm.

The acceptable level of smoothing is dependent on the scan speed.

SPEED:	up to 960 nm/min	1920 nm/min	2880 nm/min
SMOOTH:	0, 2, 3, 4 6, 8, 10 nm	0, 4, 6, 8, 10 nm	0, 6, 8, 10 nm

Guideline: 0.5 x peak width of lowest peak.


Smooth 2 nm, corresponds to ½ Peak width. Spectrum quality not compromised
Smooth 6 nm. Spectrum quality poor.
Smooth value is 10 nm. Benzene spectrum not recognizable

Smoothing influences resolution and noise: an increase in smoothing decreases the noise, but resolution suffers.
A too high degree of smoothing tends to average out the values and the fine structures of the spectrum disappear.
If smoothing is too low, spectral fine structures may be hidden by noise.
If an unacceptable value is selected, an appropriate indication is displayed. If then no other value is substituted, the spectrometer will automatically continue to operate at smoothing 0.
Note: Derivative spectra cannot be scanned at smoothing 0.

Concentration methods.

Select a smoothing factor that is as low as possible so that the peak is smoothed as little as possible. The value selected should depend on the level of noise.

Parameter	No.	Description
SPEED	13	Scanning speed in nm/min. Select the scanning speed depending on the type of sample and the desired resolution. The following can be used as a general rule: - For narrow peaks, select a low scanning speed and low degree of smoothing in order to limit the noise level. - For broader peaks, higher scanning speeds can be selected. - For derivative spectra, observe the comments given under ORDINATE MODE. Guideline values: Overview spectra — 2880; 1920 nm/min Broad peaks — 960; 480; 240 nm/min Solid and liquid samples — 120; 60; 30 nm/min Gaseous samples, spectra with higher resolution expanded spectra — 30; 15; 7.5 nm/min
STIRRER	49	Switches magnetic stirrer on and off. Option: YES NO If magnetic stirring has been switched on, place a small magnetic stirring bar in each of the cells. The arrangement is such that whilst measurement is taking place in one cell, the following cell is stirred. *Used with the 13Cell changer accessory.*
TEMP CHECK	50	Temperature measurement. Option: YES NO If TEMP CHECK = YES has been selected, a temperature sensor (Part Number B018-5227) must be in stalled. The temperature measured in the cell is included in the printout. If a temperature sensor has not been installed, select TEMP CHECK = NO.

Parameter	No.	Description
TEMPERATURE	**51**	To enter the required temperature in °C (only in combination with TEMP CHECK = YES). Range: 0.0 to 150.0 Measurement begins as soon as the temperature in the cell has reached the required temperature + 0.1 °C. For temperatures of 0.0 °C or less, the temperature is only registered and printed out together with the analytical results. **Operation with a Peltier cell holder.** To enter the required temperature of the Peltier cell holder. Range: 15.0 to 45.0
THRESHOLD	**33**	**Scan.** Threshold value for the printout of analytical data (valid only when PRINT DATA = YES). Range: 0.0 to 9999 Only data above the given threshold value is printed out. **Concentration.** Threshold value for MODE = DERIV PEAK 2. Only values above the given threshold value will be recognized as peaks.
TIME UNIT	**20**	Units for all subsequent time parameters. min: minutes s: seconds
TM CALCULATION	**76**	Calculates the theoretical melting point (T_M). *Refer to the Biochemical Application manual.*

Parameter	No.	Description
TOTAL TIME	**22**	**Total time** from the start of the method (or end of DELAY TIME) to the end of the measurement (units as selected for TIME UNIT). Range: 0.1 to 999.9 *Select the measuring time so that the end of the measurement is still within the linear portion of the curve.* ***Manual operation:*** *For CALCULATE = REGRESSION, the time is that between the start of the method and end of the measurement.* *For CALCULATE = INTERVAL, the time is that between the end of the DELAY TIME and end of the measurement.* ***Operation with Cell Changer:*** *The time from the end of the DELAY TIME to the end of the measurement.* *Within this measuring period, all samples are measured n-times consecutively.* *The number of cycles is determined by the measuring time and the INTERVAL time:* *Number of cycles = TOTAL TIME/INTERVAL + 1.* *If the result proves to be a decimal fraction, the next higher whole number is taken, e.g. 100/30 + 1 = 4.33 = 5 cycles.* *The cycle time is identical to the INTERVAL time..* *The final cycle begins at the end of the measuring time i.e. measurement ends only on completion of the final cycle.*
TRAY #	**50**	Enter the number of the tray used with the AS90/91 autosampler.
VALUE n	**8**	If the calibration curve is to be compiled from known values (e.g. from the literature values or from previous measurements), these values should be entered here. *Make sure that the entering sequence is the same as for the corresponding concentrations (parameter REF).* If the calibration curve is to be recorded, do not enter anything here. *After calibration, the absorbance values for the reference solutions are automatically entered.* *The spectrometer calculates the calibration curve from these values.*

Parameter	No.	Description
WAV.MAX	3	Upper limit of the wavelength range in nm. Value range: 190.0 to 1100.0; in steps of 0.1.
WAV. MIN	4	Lower limit of the wavelength range in nm. Range: 190.0 to 1100.0, in steps of 0.1. *If peak areas are used for the calculation (see parameter MODE), the area is calculated between the upper and lower wavelength values.* *If calculation is made via a 2nd order derivative, the derivative spectrum is recorded between the upper and lower wavelength values.*
WAVE. n	3	**Wavelength program, Oligoquant 1.** Measuring wavelength in nm. Range: 190.0 to 1100.0, in steps of 0.1. ***Note:*** *The wavelengths is run in the sequence in which they are entered (when using the ORDINATE MODE, take RAT and DIF into account).* *Enter wavelengths in decreasing sequence if possible – highest first, lowest last.* **Concentration 1.** Wavelength (n) in nm. Range: 190.0 to 1100.0, in steps of 0.1. When using MODE = ABS, only 1 wavelength can be entered. When using MODE = DELTA ABS, 2 wavelengths must be entered. When using MODE = 3 WL ANALYS, 3 wavelengths must be entered. WAVELENGTH. 1 = measuring wavelength WAVELENGTH. 2 and 3 = wavelengths for baseline correction. *See page 10-14.*
WAVELENGTH	3	The **wavelength** (nm) at which measurements are made. Range: 0 (for adjustment of cell holder) 190.0 to 1100.0 in steps of 0.1.

Appendix

A1.1 Activating SuperUser Mode

SuperUser mode gives you access to all the branches in the instrument. You activate SuperUser mode as follows:

1) Switch off the spectrometer.
2) Wait about two minutes to allow the lamps to cool down.
3) Press **[1] [5] [−]** (one, five, minus) simultaneously.
4) Keep the keys pressed and switch on the spectrometer.
5) Release the keys when the display appears.

The SuperUser mode is now active.

A1.2 Deactivating SuperUser Mode

1) Switch off the spectrometer.
2) Wait about two minutes to allow the lamps to cool down.
3) Switch on the spectrometer.

The SuperUser mode is now deactivated.

A2.1 General Information about Protect Functions

Protect functions define the right of access to methods and branches. They can only be defined in the SuperUser mode.

You can prevent access to branches and methods using the protect function. You can set the protection so that for routine sample checking only the method dedicated to that task is accessible.

Table A2-1 lists the protect functions available in order of priority, WRITE has lowest priority, ALL (full) has highest priority.

Table A2-1 Protect Functions

Protect Function	Effect	Designation
Write protection	Prevents method parameters from being written over.	WRITE
Read/Write protection	Prevents methods from being read and from being over-written.	RD/WR
Execute protection	Prevents methods from being used.	EXECUTE
Full protection	Prevents access to a branch and all of its methods.	ALL

A2.2 Setting Protect Functions

Please observe the following when setting protect functions:

- Protect functions set for a method are valid for this particular method only.
- Protect functions set for a branch are automatically valid for all the methods contained in the branch.
- If a particular method has a higher protect function priority than the branch, the method protection is valid.
- If the method has a lower priority than the branch, the branch protection is valid.

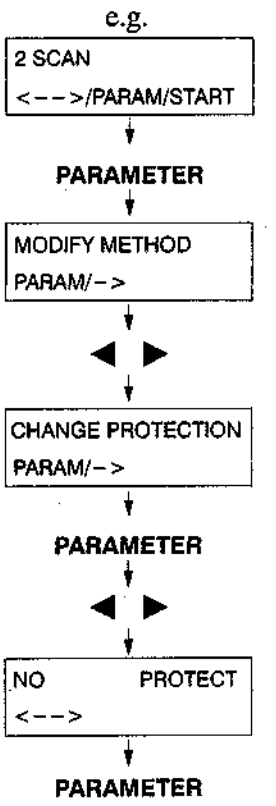
Example:

When a branch has read and write protection: all the methods in this branch have the same protection.
However, full protection can be set for individual methods, since full protection has a higher priority than read/write protection.
Write protection is not possible for individual methods since the branch has the higher priority.

Setting Method Protect Function

Set the method protect function as follows:

1) Switch on the spectrometer in SuperUser mode.
2) Select appropriate method.
3) Press **[PARAMETER]**.
4) Use the arrow keys to select CHANGE PROTECTION.
5) Press **[PARAMETER]** again.
6) Use the arrow keys to select the desired protect function.
7) Press **[PARAMETER]** to confirm the protection
   ...or...
   Press **[STOP]** to cancel.
8) Exit SuperUser mode to activate the protect function

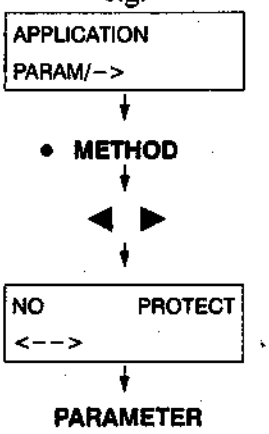


Setting Branch Protect Function

Set the branch protect function as follows:

1) Switch on the spectrometer in SuperUser mode.
2) Select the appropriate branch.

e.g.



3) Press **[•] [METHOD]**.
4) Use the arrow keys to select the required protect function.
5) Press **[PARAMETER]** to confirm the protection

   ...or...

   Press **[STOP]** to cancel.
6) Exit SuperUser mode to activate the protect function

Preventing Access to the Methods and Branches

You can prevent access to branches and methods using the protect functions.

Example

If only one particular method is to be used (without access to the parameters), set the protect functions as follows:

- Set full protection for all branches except the application branch.
- Set read/write protection for the application branch.
- Set full protection for all methods in the application branch not to be used.

For the method to be used, the read/write protection of the branch is valid.
This method can be used outside of the SuperUser mode (i.e. started), but it cannot be read or altered.

Tagged parameters continue to appear when methods are being run and may then be modified.

A3.1 Overview Of the Instrument Branches

The basic spectrometer has 6 branches

Branch	Content
Application	Analysis methods
Communication*	Interface methods
Calibration*	Methods to calibrate the spectrometer
Configuration*	To set up basic spectrometer settings
Test*	Test methods
Validation*	Validation methods

* Can only be accessed as SuperUser.
Methods can only be modified, you cannot create any new methods.

A3.2 Selecting a Branch

1) Switch on the spectrometer in SuperUser mode.

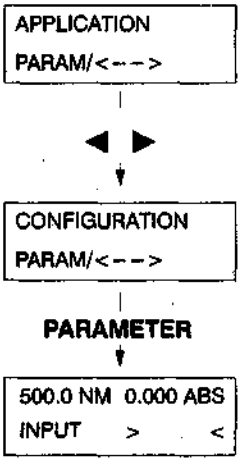
2) Press **[STOP]** until a branch header is displayed.

3) Use the arrow keys to select the required branch.

4) Press **[PARAMETER]**.

   The branch methods can then be selected (*see Section 4.2, page 4-2*).

   Can only be set in SuperUser mode.



A3.3 About the Various Branches

With branches that do not contain analytical methods, you must always press **[START]** *after making the change to make the changes effective.*

A3.4 APPLICATION - the Application Branch

This branch contains the analytical methods.

A3.5 COMMUNICATION - the Communication Branch

This branch contains a method for controlling the spectrometer via an external computer.

No.	Method	Function
29	FREERUN	Control of the spectrometer via an external computer. A precondition is that the computer should be equipped with a program compatible with the method in use, e.g. UV WinLab, or PECSS (version 3.1 or later).

A3.6 CALIBRATION - the Calibration Branch

Only for service personnel (or very experienced users). This branch contains methods for wavelength calibration of the spectrometer.

No.	Method	Function
19	0% T CALIBRATION	Switches the dark signal compensation on and off. A residual current (dark signal) flows through the detector even when there is no beam. This signal is taken into account when the dark signal function is switched on. Compensation then takes place automatically, either at the start of a method or every 10 minutes, whichever occurs first.
20	ONE WAVEL. CALIB.	Wavelength calibration with one peak. To check the calibration, record the spectrum of a wavelength standard and compare with that recorded by the spectrometer. If they do not correlate, the spectrometer should be recalibrated. Parameters: 0 nm PEAK — Internal calibration at 0 nm. D2 PEAK — Internal calibration at 656.1 nm. SPEC PEAK — Calibration using an external wavelength standard, e.g. holmium oxide. OLD PEAK — Measured wavelength of the external standard. NEW PEAK — Actual wavelength of the external standard.
21	TWO WAVEL. CALIB.	Wavelength calibration with two peaks. To check the calibration, record the spectrum of a wavelength standard and compare with that recorded by the spectrometer. If they do not correlate, the spectrometer should be recalibrated. Parameters: AUTO PEAK — Internal calibration at 0.0 and 656.1 nm. SPEC PEAK — Calibration using an external wavelength standard, e.g. holmium oxide. OLD PEAK n — Measured wavelength of the peak. NEW PEAK n — Actual wavelength of the peak.

A3.7 CONFIGURATION - the Configuration Branch

This branch contains methods for configuration of the spectrometer to the individual requirements of the user.

No.	Method	Function
1	HELP CONFIG	Level and language of help messages. Parameters: LEVEL Extent LANGUAGE Language
2	AS90/91 CONFIG	Setting for the AS-90/91 (sample locations, upper and lower limits for the sampler arm, etc.). Details are contained in the AS-90/91 User's Manual.
4	COMM. CONFIG	Protocol for the RS 232 interface for use with a PC. The default parameter values are for use with PECSS and an Epson PC. Default values: Port Enable: No / ETXT-Character: 015 Respond: Prompt / Prompt-Character: 021 Baud Rate: 4800 / Break-Character: 043 Bits/Character: 8 / Erase-Character: 010 Stopbit: 1 / Kill-Character: 010 Parity: none / Range: 2 Terminator: CRLF
5	PORT USAGE CONF.	Usage of the RS 232 interface. The parameter MAIN PORT determines, whether a printer or a PC can be connected to the RS 232 interface: MAIN PORT / 1st RS-232 / 2nd RS-232 PRINTER / printer / PC COMPUTER / PC / printer *After a full reset the value is set to* PRINTER. *To facilitate administration, set* MAIN PORT= PRINTER *and connect the printer to the 1st RS 232 interface, the PC to the 2nd RS 232 interface.* If one interface fails, change the method to switch the interface usage.

No.	Method	Function
6	PRINTER CONFIG	Printer Output. Parameters: PRINTER ON? Switches output to the printer on and off. PRINTER Selects the printer: EPSON LQ EPS. EX/FX + BIDIRECTIONAL GAP Sets the gap between two pages. COLOR ON? Switches color printing on and off (only for color printers). PERFORATION Skip over Perforation. PLOT HEADER Header printed at the start of each plot.
7	ACCESSORY CONFIG	Accessory mode (for spectrometers without accessory board). Parameters: ACCESSORY ON? Switches accessory mode on and off. CELL To select the type of cell changer. NO is no cell changer. CELL5, CELL6, CELL8, CELL9, CELL13 is for cell changer type. SIPPER To select the type of sipper. VASI, PESI SI = sipper, VA = vacuum, PE = peristaltic. AS-90/91 autosampler
8	USER CONFIG	Switches from single to double beam mode; switches background correction on and off. Parameters: BEAM DB Double beam BEAM SBR Single reference beam BEAMSBS Single sample beam BASELINE CORR? YES Background correction on BASELINE CORR? NO Background correction off *Note: When using single beam mode, operate only with ordinate mode %T.*

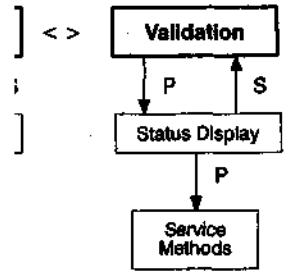
No.	Method	Function	
9	FACTORY CONFIG	Calibration peaks offsets and filter change points.	
		Parameters:	
		0NM OFFSET	Only for service personnel. Default: 0.0 nm
		ABS FACT	Only for service personnel. Default: 1.0
		D2 OFFSET	Only for service personnel. Default: 0.0 nm
		FILTER n	Wavelengths for filter change (filter 2-7) Default: filter 2 = 830.0 nm filter 3 = 683.0 nm filter 4 = 558.0 nm filter 5 = 420.0 nm filter 6 = 383.0 nm filter 7 = 326.0 nm (lamp change point)

A3.8 VALIDATION - the Validation Branch

The methods in this branch are reserved for customer service. The methods are used to test that the spectrometer's performance lies within specification.

A3.9 TEST - the Test Branch

These methods are for use by Perkin-Elmer Service only.



M: [METHOD] n [ENTER]

P: [PARAMETER]

S: [STOP]

< >: Arrow keys

A4.1 Enzymatic Analytical Procedures

Enzyme analysis makes use of enzyme controlled reactions to determine a substance. These procedures are used especially in clinical chemistry and food chemistry.

There are two methods of enzymatic analysis:

- The determination of enzyme concentration or enzyme activity: enzyme kinetic measurement. For example: the determination of enzymes in blood serum.
- The determination of the concentration of a substrate: substrate kinetic measurement. For example: the determination of components in food, e.g. alcohol in wine.

The basis of both methods is the conversion of a substrate into a product whereby the enzyme acts as a catalyst.

The reaction can be followed photometrically: an added coenzyme (e.g. NADH) is oxidized or reduced in the course of the reaction and the resulting change in absorbance measured. Or, the substrate or product may be photoactive and the absorbance will change with the concentration.

In enzyme kinetics, the substrate is added in excess and the reaction rate (as dA/dt) measured. With excess substrate, it is constant and directly proportional to enzyme activity.

In substrate kinetics, the substrate reacts completely. The substrate concentration can then be calculated from the change in absorbance (ΔA).

A4.2 Enzyme Kinetics

In enzyme kinetics, the enzyme activity of a sample solution is determined: the sample solution containing enzyme (e.g. serum) is reacted with a high excess of substrate.

The substrate is converted to product by the enzyme, the rate of the reaction can be followed photometrically and is a direct measure of the enzyme activity.

(Enzyme activity is given as International Units U:
1U = the enzyme activity required to convert 1 µmole of substrate per minute, under optimal conditions.)

The following reaction can be assumed for the conversion of substrate S into product P:

$$S + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} S{-}{-}E \xrightarrow{k_3} P + E$$

where:

E = enzyme and k = reaction rate constant.

The rate of reaction $S + E$ ----------> $P + E$ is governed by the Michaelis-Menten equation:

$$v = k_3 \cdot \frac{c_{0E} \cdot c_S}{k_M \cdot c_S}$$

with $v = \frac{dP}{dt} = -\frac{dS}{dt} = \frac{dA}{dt}$ = Reaction Rate

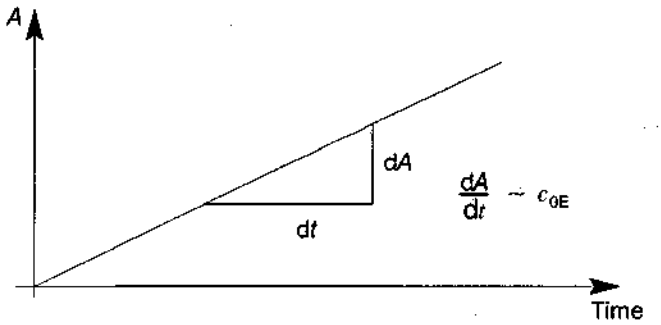
and $k_M = \frac{k_2 + k_3}{k_1}$ = Michaelis-Constant

Due to the large excess of substrate there is a limiting case:

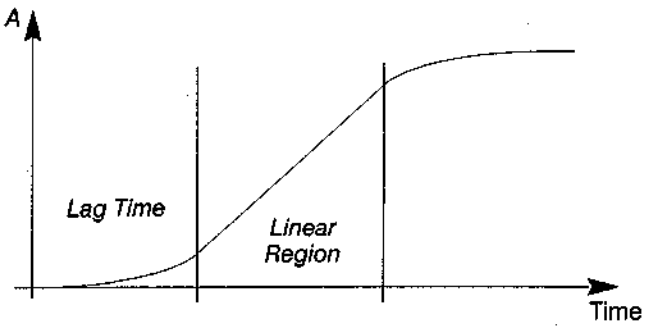
$c_S \gg k_M$ and hence

$v = k_3 \cdot c_{0E}$ is valid.

Thus, the reaction rate is constant (no consumption of enzyme) and directly proportional to enzyme concentration or enzyme activity. Hence, the enzyme activity of a solution can be directly determined by measuring the reaction rate.



In practice, the course of the curve obtained can deviate from the ideal form: it becomes linear only after a certain lag time and flattens out towards the end. In such a case, only the linear region of the curve is used for calculating the reaction rate.



A4.3 Substrate Kinetics

In substrate kinetics the substrate concentration of a sample solution is determined via enzyme controlled reactions. The advantages of such a process are:

- High specificity, i.e. only one substrate is converted. This avoids the necessity of complex sample preparation.
- A quicker reaction, with measuring times of only 3–30 minutes.

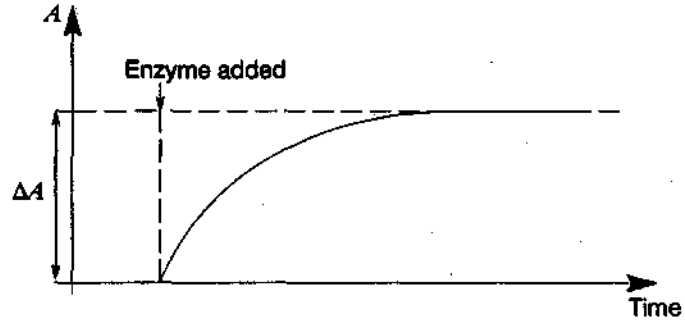
In the course of the reaction, the substrate is converted to product and the reaction can be followed photometrically. The reaction is started by the addition of enzyme and proceeds relatively quickly until a state of equilibrium is attained. The substrate has been converted by this time and the absorbance does not alter any more.

The measured difference in absorbance (ΔA) is directly proportional to the substrate concentration:

$$c_{Sub} = f \cdot \Delta A$$

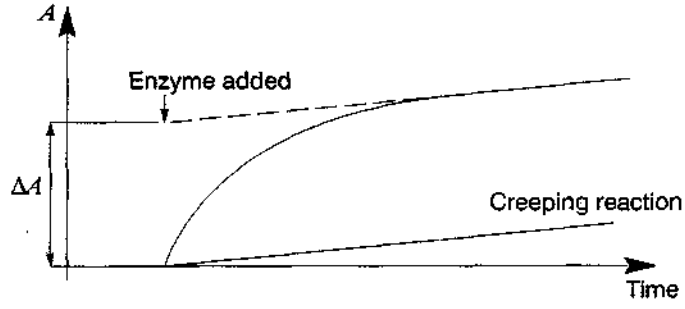
where:

c_{Sub}: is the substrate concentration
f: is the concentration factor
ΔA: is the measured difference in absorbance



The course of the reaction can deviate from the ideal described above: creeping reactions can take place and the absorbance can hence increase even after the substrate reaction has been completed.

The end point of such a reaction is reached when the slope of the curve remains constant. The actual end point can then be determined by extrapolation.



Translations of Warnings

Translations of Warnings

This annex contains translations of the *numbered* warnings used in this handbook.

W01.01

WARNING

Warning
We use the term WARNING to inform you about situations that could result in ***personal injury*** *to yourself or other persons.*
Details about these circumstances are in a box like this one.

D

Warning (Warnung)
Bedeutet, daß es bei Nichtbeachten der genannten Anweisung zu einer ***Verletzung*** *des Benutzers kommen kann.*

DK

Warning (Advarsel)
Betyder, at brugeren kan blive ***kvæstet****, hvis anvisningen ikke overholdes.*

E

Warning (Peligro)
Utilizamos el término ***WARNING*** *(PELIGRO) para informarle sobre situaciones que pueden provocar* ***daños personales*** *a usted o a otras personas.*
En los recuadros como éste se proporciona información sobre este tipo de circunstancias.

F

Warning (Danger)
Nous utilisons la formule ***WARNING*** *(DANGER) pour avertir des situations pouvant occasionner des* ***dommages corporels*** *à l'utilisateur ou à d'autres personnes.*
Les détails sur ces circonstances sont données dans un encadré semblable à celui-ci.

I

Warning (Pericolo)
Con il termine ***WARNING*** *(PERICOLO) vengono segnalate situazioni che potrebbero provocare* ***incidenti alle persone****. Troverete informazioni su tali circostanze in un riquadro come questo.*

NL

Warning (Waarschuwing)
Betekent dat, wanneer de genoemde aanwijzing niet in acht wordt genomen, dit kan leiden tot ***verwondingen*** *van de gebruiker.*

P

Warning (Aviso)
Significa que a não observância da instrução referida poderá causar um ***ferimento*** *ao usuário.*

C01.01

Caution

We use the term CAUTION to inform you about situations that could result in ***serious damage to the instrument*** *or other equipment.*
Details about these circumstances are in a box like this one.

D

Caution (Achtung)

Bedeutet, daß die genannte Anleitung genau befolgt werden muß, um einen ***Geräteschaden*** *zu vermeiden.*

DK

Caution (Bemærk)

Dette betyder, at den nævnte vejledning skal overholdes nøje for at undgå en ***beskadigelse af apparatet****.*

E

Caution (Advertencia)

Utilizamos el término ***CAUTION*** *(ADVERTENCIA) para advertir sobre situaciones que pueden provocar* ***averías graves en este equipo*** *o en otros. En recuadros éste se proporciona información sobre este tipo de circunstancias.*

F

Caution (Attention)

Nous utilisons le terme ***CAUTION*** *(ATTENTION) pour signaler les situations susceptibles de provoquer de* ***graves détériorations de l'instrument*** *ou d'autre matériel.*
Les détails sur ces circonstances figurent dans un encadré semblable à celui-ci.

I

Caution (Attenzione)

Con il termine ***CAUTION*** *(ATTENZIONE) vengono segnalate situazioni che potrebbero arrecare* ***gravi danni allo strumento*** *o ad altra apparecchiatura.*
Troverete informazioni su tali circostanze in un riquadro come questo.

NL

Caution (Opgelet)

Betekent dat de genoemde handleiding nauwkeurig moet worden opgevolgd, om ***beschadiging van het instrument*** *te voorkomen.*

P

Caution (Atenção)

Significa que a instrução referida tem de ser respeitada para evitar a ***danificação do aparelho.***

W01.03

WARNING

Explosive Atmosphere
This instrument is not designed for operation in an explosive atmosphere.

(D)

Explosionsfähige Atmosphären
Das Gerät darf nicht in explosionsfähigen Atmosphären betrieben werden!

(DK)

Eksplosive omgivelser
Apparatet må ikke anvendes i eksplosive omgivelser!

(E)

Atmósfera explosiva
Este aparato no ha sido diseñado para utilizarlo en atmósferas explosivas.

(F)

Atmosphère explosive
Cet instrument n'est pas conçu pour fonctionner dans une atmosphère explosive.

(I)

Atmosfera esplosiva
Questo strumento non è adatto per l'uso in atmosfera esplosiva.

(NL)

Explosiegevaarlijke omgevingen
Het instrument mag niet in een explosiegevaarlijke omgeving worden gebruikt!

(P)

Atmosferas explodíveis
O aparelho não pode ser utilizado em atmosferas explodíveis!

W02.01

WARNING

Electrical Hazard
Any interruption of the protective conductor inside or outside the instrument or disconnection of the protective conductor (earth/ground) terminal is likely to make the instrument dangerous.
Intentional interruption is prohibited.

(D)

Gefährdung durch Elektrizität
Das Gerät muß zum Betrieb immer geerdet sein.
Auf keinen Fall die Schutzleiter im Gerät oder in der Netzzuleitung trennen oder entfernen.

(DK)

Fare på grund af elektricitet
Apparatet skal altid være jordet.
Man må under ingen omstændigheder skille eller fjerne jordlederen inde i apparatet eller i strømledningen.

(E)

Peligro eléctrico
Cualquier interrupción del conductor de protección dentro o fuera del aparato, o la desconexión del terminal del mismo (toma de tierra) podrían ocasionar serios peligros al usar el equipo.
Prohibida la interrupción intencionada.

(F)

Risque d'électrocution
Toute interruption du conducteur de protection à l'intérieur ou à l'extérieur de l'instrument, ou déconnexion du raccord du conducteur de protection (terre) peut rendre l'instrument dangereux. Il est interdit d'interrompre volontairement ce conducteur.

(I)

Pericolo: elettricità
Qualsiasi interruzione della protezione del conduttore all'interno o all'esterno dello strumento, o lo scollegamento del terminale (di terra/massa) del conduttore di protezione possono rendere pericoloso lo strumento.
È' vietato provocare volontariamente queste interruzioni.

(NL)

Risico's door elektriciteit
Het instrument moet voor de werking altijd geaard zijn. In geen geval mag de aarding van het instrument of de netvoeding worden onderbroken of worden verwijderd.

(P)

Perigo por electricidade
Para a operação o aparelho tem de estar sempre ligado à terra. De forma alguma separar ou retirar os condutores de protecção à terra no aparelho ou no cabo de alimentação da rede.

W02.02

WARNING

Lethal voltages are present in the instrument

- *Even with the power switch OFF, line power voltages can still be present within the instrument.*
- *When the instrument is connected to line power, terminals may be live, and opening covers or removing parts (except those to which access can be gained by hand) is likely to expose live parts.*
- *Capacitors inside the instrument may still be charged even if the instrument has been disconnected from all voltage sources.*

(D)

Gefährliche Spannung im Gerät

- *Auch in ausgeschaltetem Zustand kann an einigen Stellen im Gerät Netzspannung anliegen, wenn das Gerät am Stromnetz angeschlossen ist.*
- *Auch bei ausgeschaltetem Gerät und getrennter Netzverbindung können Kondensatoren im Gerät noch mit gefährlicher Spannung geladen sein.*

(DK)

Farlig spænding i apparatet – fare for kvæstelser

- *Også i slukket tilstand kan der være netspænding nogle steder i apparatet, hvis apparatet er tilsluttet til strømnettet.*
- *Selv når apparatet er slukket, og strømforbindelsen er afbrudt, kan kondensatorerne i apparatet være ladet med farlig spænding.*

(E)

En el aparato existen voltajes letales

- *Incluso con el interruptor desconectado, puede haber voltaje dentro del equipo.*
- *Cuando el instrumento se encuentre conectado a la red eléctrica, los terminales pueden estar bajo corriente y éstos quedar expuestos al abrir las cubiertas o al extraer componentes (exceptuando aquellos a los cuales se puede acceder con la mano).*
- *Los condensadores internos del aparato pueden permanecer cargados incluso cuando el aparato haya sido desconectado del voltaje de la línea.*

(F)

Présence de tensions mortelles dans l'instrument

- *Même lorsque l'interrupteur de puissance est sur ARRET, des tensions de secteur peuvent encore être présentes dans l'instrument.*
- *Lorsque l'instrument est relié au secteur, les raccords peuvent être sous tension, et des parties sous tension peuvent être découvertes en ouvrant des capots ou en retirant des pièces (à l'exception de celles auxquelles il est possible d'accéder manuellement).*
- *Les condensateurs contenus dans l'instrument peuvent encore être chargés, même si l'instrument a été déconnecté de toutes les sources de tension.*

W02.02

WARNING

Lethal voltages are present in the instrument

- *Even with the power switch OFF, line power voltages can still be present within the instrument.*
- *When the instrument is connected to line power, terminals may be live, and opening covers or removing parts (except those to which access can be gained by hand) is likely to expose live parts.*
- *Capacitors inside the instrument may still be charged even if the instrument has been disconnected from all voltage sources.*

I

Nello strumento sono presenti tensioni mortali

- *Anche se l'interruttore di alimentazione è in posizione OFF, la tensione di linea può essere ancora presente all'interno dello strumento.*
- *Quando lo strumento è collegato alla rete di alimentazione i terminali possono essere sotto tensione; aprendo le calotte di protezione o rimuovendo alcune parti (ad eccezione di quelle raggiungibili con la mano) è possibile esporre altre parti sotto tensione.*
- *Anche se lo strumento è stato scollegato da tutte le fonti di tensione, i condensatori al suo interno possono essere ancora carichi.*

NL

Gevaarlijke spanningen in het instrument – gevaar van letsel

- *Ook in uitgeschakelde toestand kan, wanneer het instrument op de netvoeding is aangesloten, op sommige plaatsen in het instrument netspanning staan.*
- *Ook bij een uitgeschakeld instrument en een onderbroken netvoeding kunnen condensatoren in het instrument nog een gevaarlijke lading bevatten.*

P

Tensão perigosa no aparelho – perigo de ferimento

- *Mesmo desligado, o aparelho poderá ainda ter tensão de rede em alguns pontos enquanto estiver ligado à rede de corrente.*
- *Mesmo com o aparelho desligado e a ligação à rede de corrente interrompida, os condensadores dentro do aparelho ainda poderão ter ums tensão perigosa aplicada.*

Index

Index

A

ABS, 10-14, 10-19
Accessory, 10-1
  configuration, A3-6
  operation with, 6-1
  parameters, 6-7
  requirements, 6-2
  spectrometer without ... board, 6-3
  type, 6-1
  with methods, 6-2
Alignment
  cell holder, 2-6
  microcell, 2-9
Analysis Procedure, 5-3
Application
  branch, A3-3
  minimum volume ..., 2-9
AS-90/91, 6-9, 10-1
  configuration, A3-5
Auto Method, 10-1
Auto Purge, 10-1
Autopurge, 6-9

B

Back Corr, 10-2
Background Correction, 5-2, 8-1, 10-2
  manual setting, 3-3
  with cell changers, 6-5
Baseplate, 2-4
Baud rate, for PC, A3-5
Beer-Lambert Law, 10-11, 10-12
Beer-Lambert Law, 8-2
Blank, 10-3
Branch
  application, A3-3
  calibration, A3-4
  communication, A3-3
  configuration, A3-5
  directory, 4-16
  protect function, A2-4
  selecting, A3-2
  test, A3-7
  validation, A3-7
Branches, overview, A3-1

C

Calc.Wav 1, 10-3
Calc.Wav 2, 10-3
Calculate, 10-4, 10-12, 10-26
  interval, 10-4
  regression, 10-4
Calibration
  branch, A3-4
  curve, 10-8, 10-26
    deleting a point from, 5-25
    processing, 5-24
Care
  daily, 7-1
  of the instrument, 7-1
Cell, 2-9, 10-1
  centre height, 2-7
  changer
    13cell, 6-8
    5cell, 6-7
    6cell, 6-7
    8cell, 6-7
    9cell, 6-7
  holder, 2-4
    alignment, 2-6
    installation, 2-5
  lifter for short cells, 2-4
  part numbers, 2-9
  pathlength, 2-9
  use and care of, 7-2
Cell 1-7, 10-5
Cell 5, 10-5
Cell 6, 10-5
Cell 8, 10-5
Cell 8-13, 10-5
Cell 9, 10-5
Chemically Reactive Samples, 8-3
Chng Const, 10-5
Cleaning
  sample compartment, 7-1
  sample compartment window, 7-1, 7-3
Clock
  internal, 5-41
  realtime, 5-41
Cold Solutions, 7-2
Communication
  branch, A3-3
  configuration, A3-5

Compressed gases, safety information, S-7
Conc, 10-19
  factor, 10-6
  unit, 10-6
Concentration Method, 5-12
  concentration 1
    calibration curve, 5-15
    create, 5-14
    measurement wavelength(s), 5-13
    sample measurement, 5-16
    summary, 5-12
  concentration 2
    2nd derivative, 5-19
    calibration curve, 5-22
    create, 5-21
    measurement wavelengths, 5-19
    peak areas, 5-19
    sample measurement, 5-23
    summary, 5-18
    threshold, 5-20
  with cell changers, 6-6
Configuration
  accessory, A3-6
  AS-90/91, A3-5
  branch, A3-5
  communication, A3-5
  factory, A3-7
  help, A3-5
  port usage, A3-5
  printer, A3-6
  user, A3-6
Connector, panel, 1-1
Copying
  method, 4-14
  method parameters, 4-13
Cor, 10-1, 10-19, 10-20
Creep
  cycle, 10-7
  time, 10-7
Creeping reactions, A4-5
Cur Fit, 10-8
Curve
  calibration, 5-12, 5-15, 5-22, 5-24, 10-26
  changing the type of ... fit, 5-24
  type of ... fit, 10-8
Cycle Time, 10-8
Cycles, 10-8

D

D1, 10-19
D2, 10-19
D3, 10-19
D4, 10-19
Daily Care, 7-1
Date/Time, 5-40
Default Methods, 4-3
Delay Time, 6-9, 10-9
Deleting, a method, 4-8
Delta ABS, 10-14
Deriv 2 Fix, 10-15, 10-16
Deriv 2 Peak, 10-15, 10-16
Derivative, second, 5-19
Dif, 10-1, 10-19, 10-20
Dil Factor, 10-9
Directory
  branch, 4-16
  printing, 4-16
  spectrometer, 4-16
Display, 1-1, 1-4
  initialization, 2-2
  standby, 2-2
  symbols, 4-6
Divisor, 10-9, 10-10
Double Index of Refraction, 8-3

E

Editing Methods, 4-4
Electricity, safety information, S-4
End Time, 10-10
Enz
  factor, 10-10
  unit, 10-11
Enzyme Kinetics, 5-26, A4-2
  activity, 5-26, A4-2
    international unit, A4-2
  blank value, 5-28
  enzymatic analytical procedures, A4-1

F

Factor, 10-11
  ... n, 10-12
  concentration, 10-6
  dilution, 10-9
  enzyme, 10-10
Features, 1-1
Filter change points, A3-7
First Sample #, 10-12
Form Feed, 1-3

G

Gases, safety information, S-7
Graphics Plot, 10-12
Grid, 10-12

H

Help, A3-5
  key, 4-17
  key combinations, 4-17
  text, 4-17

I

Installing, cell holder, 2-5
Instrument, care of the ..., 7-1
Interface Failure, A3-5
Interference Pattern, 8-3
Interrupting the Measurement, 5-3

Interval, 10-12, 10-13
Interval Time, 5-34

K

Key
  arrow, 1-2
  back corr, 1-2
  ce, 1-2
  combinations, 1-3
  enter, 1-2
  goto L@, 1-2, 1-3
  help, 1-2, 1-3
  method, 1-2, 1-3
  minus, 1-2
  parameter, 1-2, 1-3
  point, 1-2
  start, 1-2
  stop, 1-2
Keyboard, 1-1

L

Lag Time, 5-34, 10-13
Lamp, 10-13
Lamp Compartment, 1-1
Lifter, 2-4
Line Feed, 1-3
Line Type, 10-13
Lower Wavelength Limits, of solvents, 8-5

M

Mark for Copy, 4-13
Measurement Wavelengths, 5-19
  2nd derivative, 5-19
  peak areas, 5-19
  peak heights, 5-12, 5-13
Method
  checking, 4-12
  concentration, 5-12
  copying, 4-14
  copying ... parameters, 4-13
  creating a ... file, 4-9
  creating a new ..., 4-9
  deleting, 4-8
  editing, 4-4
  modify, 4-5
  name, 4-11
  overview, 5-1
  overwriting, 4-10
  printing, 4-15
  procedure, 5-2
  protect function, A2-3
  types, 5-1
Michaelis constant, A4-2
Michaelis-Menten Equation, A4-2
Microcell, 2-9
  alignment, 2-9
  liquid height, 2-9
  minimum volume application, 2-9
MMass
  dA, 10-16
  dC, 10-16
  dG, 10-17
  dN, 10-17
  dT, 10-17
Mode, 10-14, 10-15, 10-16
  ordinate, 10-19, 10-20

N

New from Mark, 4-14
New Method, creating a ..., 4-9
Number of
  dA, 10-17
  dC, 10-17
  dG, 10-17
  dT, 10-17
  N, 10-17

O

Oligo, 10-18
Oligo Method
  oligoquant 1, 5-36
  oligoquant 2, 5-36
Oper.ID, 10-18
Optimization, 6-9
Ord.Max, 10-18
Ord.Min, 10-18
Ordinate Mode, 10-19, 10-20
Overlay, 10-20
Overview
  branches, A3-1
  methods, 5-1

P

Parameter
  changing, 4-6
  descriptions, 10-1–10-28
  numbers, 10-1–10-28
  tagging, 4-7
Part numbers, cell, 2-9
Pathlength, of cells, 2-9
Peak
  areas, 5-19, 10-15
  deriv 2, 10-15, 10-16
  heights, 5-12, 5-13
Peristaltic Sipper, 6-9
Personal Computer
  operation with, A3-5
  RS-232 interface, A3-5
Photoactive Samples, 8-3
Plot Refs, 10-20
Polarizing Samples, 8-3
Port usage, A3-5
Postrun Kinetics, 5-34, 10-20
Power switch, 1-1
Pressure buildup in cells, 7-3

Print
branch, directory, 1-3
current values, 1-3
data, 10-20
directory, 4-16
method, 4-15
directory, 1-3
information, 1-3
parameters, 1-3
Peltier temperature, 1-3
refs, 10-21
status, 1-3
using help key combinations, 4-17
Printer
configuration, A3-6
output configuring, A3-6
Protect Function, A2-1
execute, A2-1
for branches, A2-4
for methods, A2-3
full, A2-1
preventing access to methods and branches, A2-5
read/write, A2-1
setting, A2-2
write, A2-1

Q

Quick Sample Measurement, 3-4

R

Radiation beam, 2-7
Rat, 10-1, 10-19, 10-20
Ref n, 10-21
Reference Solution, remeasuring, 5-24
Refs, 10-21
Reset, 3-5
full, 1-3
Response, 10-21
Return, 6-9
to standby, 5-42
Rev. Time, 6-9
RS 232 interface
use of, A3-5
use with PC, A3-5

S

Safety Information, S-3
Salt, 10-21
Sample Compartment, 1-1
window, 7-1, 7-3
cleaning, 7-1, 7-3
Sample ID, 10-22
Sample Measurement, 5-2
Sample Volume, minimum in microcell, 2-9
Samples
/Batch, 10-22
chemically reactive, 8-3
not governed by Beer-Lambert law, 8-2
photoactive, 8-3
polarizing, 8-3
thin-film, 8-3
unusual, 8-2
volatile, 8-2
Sampling Time, 6-9, 10-22
Scale, 10-22
Scan, 5-8
Select
branch, A3-2
default method, 1-3, 4-3
method, 4-2
previous parameter, 1-3
tag, 1-3
Self Test, 5-43
Seq n, 10-22
Sequence Length, 10-22
Shutdown, 2-3
Sipper, 6-9, 10-1
Slit, 10-22
Smooth, 10-23
Solvent
lower wavelength limits of ..., 8-5
properties, 8-4
Spectrometer, directory, 4-16
Speed, 10-24
Spilled Materials, 7-1
Standby
display, 2-2
return to, 5-42
Startup, 2-2
Stirrer, 6-8, 10-24
Substrate Kinetics, 5-30, A4-4
blank value, 5-33
procedure with cell changer, 5-32
Super User, A1-1
activating, A1-1
deactivating, A1-1
mode, 1-3

T

Tagged Parameters, 5-2
Tagging, 10-2
parameters, 4-7
symbols, 4-7
type of ..., 4-7
Temp Check, 10-24
Temperature, 8-3, 10-25
Peltier cell holder, 10-25
sensor, 10-24
Test, branch, A3-7
Thin-film Samples, 8-3
Threshold, 5-20, 10-25
Time Drive, 5-6
Time Unit, 10-25
TM Calculation, 10-25
Total Area, 10-15
Total Time, 5-34, 10-26

Toxic fumes, safety information, S-7
Tray #, 10-26
Tube ports, 2-5

U

Unit
  concentration, 10-6
  enzyme, 10-11
  time, 10-25
Unusual Samples, 8-2
User, configuration, A3-6
UV radiation, S-7

V

Validation, branch, A3-7
Value n, 10-26
Volatile Samples, 8-2

W

Wakeup, 5-42
Warnings, translations of ..., T-1
Waste, disposing of, S-6
Wav.Max, 5-19, 10-27
Wav.Min, 5-19, 10-27
Wave n, 10-27
Wavelength, 10-27
  manual setting, 3-2
Wavelength Program, 5-10

Symbols

of Refs, 10-1
Wavelengths, 10-1
%T, 10-19

Numbers

3WL Analys, 10-14